

20273 BE - 5130X 10)



B11

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : <b>C07K</b>		A2	(11) International Publication Number: <b>WO 00/55180</b>						
			(43) International Publication Date: 21 September 2000 (21.09.00)						
(21) International Application Number: <b>PCT/US00/05918</b>		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).							
(22) International Filing Date: 8 March 2000 (08.03.00)									
(30) Priority Data: 60/124,270 12 March 1999 (12.03.99) US									
(71) Applicants (for all designated States except US): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US). ROSEN, Craig, A. [US/US]; 22400 Rolling Hill Road, Laytonsville, MD 20882 (US).									
(72) Inventor; and (75) Inventor/Applicant (for US only): RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Laytonsville, MD 20882 (US).		Published Without international search report and to be republished upon receipt of that report.							
(74) Agents: WALES, Michele, M. et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).		<table border="1"> <tr> <th colspan="2">Patent Dept. (TR-E) - LITERATUR</th> </tr> <tr> <td>Case / Interne Nummer</td> <td>Erfasst am</td> </tr> <tr> <td>20223 22 5130</td> <td>08.11.01 764</td> </tr> </table>		Patent Dept. (TR-E) - LITERATUR		Case / Interne Nummer	Erfasst am	20223 22 5130	08.11.01 764
Patent Dept. (TR-E) - LITERATUR									
Case / Interne Nummer	Erfasst am								
20223 22 5130	08.11.01 764								

(54) Title: HUMAN LUNG CANCER ASSOCIATED GENE SEQUENCES AND POLYPEPTIDES

(57) Abstract

This invention relates to newly identified lung or lung cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "lung cancer antigens", and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such lung cancer antigens for detection, prevention and treatment of disorders of the lung, particularly the presence of lung cancer. This invention relates to the lung cancer antigens as well as vectors, host cells, antibodies directed to lung cancer antigens and recombinant and synthetic methods for producing the same. Also provided are diagnostic methods for diagnosing and treating, preventing and/or prognosing disorders related to the lung, including lung cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of lung cancer antigens of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						



## Human Lung Cancer Associated Gene Sequences and Polypeptides

### 5 *Field of the Invention*

This invention relates to newly identified lung or lung cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "lung cancer antigens," and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such lung cancer antigens for detection,  
10 prevention and treatment of disorders of the lung, particularly the presence of lung cancer. This invention relates to the lung cancer antigens as well as vectors, host cells, antibodies directed to lung cancer antigens and recombinant and synthetic methods for producing the same. Also provided are diagnostic methods for diagnosing and treating, preventing and/or  
15 prognosing disorders related to the lung, including lung cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of lung cancer antigens of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.

### 20 *Background of the Invention*

Cell growth is a carefully regulated process which responds to specific needs of the body. Occasionally, the intricate, and highly regulated controls dictating the rules for cellular division break down. When this occurs, the cell begins to grow and divide independently of its homeostatic regulation resulting in a condition commonly referred to as cancer. In fact,  
25 cancer is the second leading cause of death among Americans aged 25-44.

Lung cancer is the primary cause of cancer death among both men and women in the U.S., with an estimated 172,000 new cases being reported in 1994. The five-year survival rate among all lung cancer patients, regardless of the stage of disease at diagnosis, is only 13%. This contrasts with a five-year survival rate of 46% among cases detected while the  
30 disease-is still localized. However, only 16% of lung cancers are discovered before the disease has spread.

Early detection is difficult since clinical symptoms are often not seen until the disease has reached an advanced stage. Currently, diagnosis is aided by the use of chest x-rays, analysis of the type of cells contained in sputum and fiberoptic examination of the bronchial passages. Treatment regimens are determined by the type and stage of the cancer, and include  
5 surgery, radiation therapy and/or chemotherapy. In spite of considerable research into therapies for the disease, lung cancer remains difficult to treat.

Accordingly, there remains a need in the art for improved vaccines, treatment methods and diagnostic techniques for lung cancer. There is a need for factors that regulate activation, and differentiation of normal and abnormal cells. There is a need for identification  
10 and characterization of such factors that modulate activation and differentiation of lung cells, both normally and in disease states. In particular, there is a need to isolate and characterize additional molecules that mediate apoptosis, DNA repair, tumor-mediated angiogenesis, genetic imprinting, immune responses to tumors and tumor antigens and, among other things, that can play a role in detecting, preventing, ameliorating or correcting dysfunctions or  
15 diseases related to the lung.

### *Summary of the Invention*

The present invention includes isolated nucleic acid molecules comprising, or  
20 alternatively, consisting of, a lung and/or lung cancer associated polynucleotide sequence disclosed in the sequence listing (as SEQ ID Nos:1 to 443) and/or contained in a human cDNA clone described in Tables 1, 2 and 5 and deposited with the American Type Culture Collection ("ATCC"). Fragments, variant, and derivatives of these nucleic acid molecules are also encompassed by the invention. The present invention also includes isolated nucleic acid  
25 molecules comprising, or alternatively consisting of, a polynucleotide encoding a lung or lung cancer polypeptide. The present invention further includes lung and/or lung cancer polypeptides encoded by these polynucleotides. Further provided for are amino acid sequences comprising, or alternatively consisting of, lung and/or lung cancer polypeptides as disclosed in the sequence listing (as SEQ ID NOS: 444 to 886) and/or encoded by a human  
30 cDNA clone described in Tables 1, 2 and 5 and deposited with the ATCC. Antibodies that bind these polypeptides are also encompassed by the invention. Polypeptide fragments, variants, and derivatives of these amino acid sequences are also encompassed by the

invention, as are polynucleotides encoding these polypeptides and antibodies that bind these polypeptides. Also provided are diagnostic methods for diagnosing and treating, preventing, and/or prognosing disorders related to the lung, including lung cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of lung cancer antigens of the invention.

### *Detailed Description*

#### Tables

Table 1 summarizes some of the lung cancer antigens encompassed by the invention (including contig sequences (SEQ ID NO:X) and the cDNA clone related to the contig sequence) and further summarizes certain characteristics of the lung cancer polynucleotides and the polypeptides encoded thereby. The first column shows the "SEQ ID NO:" for each of the 443 lung cancer antigen polynucleotide sequences of the invention. The second column provides a unique "Sequence/Contig ID" identification for each lung and/or lung cancer associated sequence. The third column, "Gene Name," and the fourth column, "Overlap," provide a putative identification of the gene based on the sequence similarity of its translation product to an amino acid sequence found in a publicly accessible gene database and the database accession no. for the database sequence having similarity, respectively. The fifth and sixth columns provide the location (nucleotide position nos. within the contig), "Start" and "End", in the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred ORF shown in the sequence listing as SEQ ID NO:Y. The seventh and eighth columns provide the "% Identity" (percent identity) and "% Similarity" (percent similarity), respectively, observed between the aligned sequence segments of the translation product of SEQ ID NO:X and the database sequence. The ninth column provides a unique "Clone ID" for a cDNA clone related to each contig sequence.

Table 2 summarizes ATCC Deposits, Deposit dates, and ATCC designation numbers of deposits made with the ATCC in connection with the present application.

Table 3 indicates public ESTs, of which at least one, two, three, four, five, ten, fifteen or more of any one or more of these public EST sequences are optionally excluded from certain embodiments of the invention.

Table 4 lists residues comprising antigenic epitopes of antigenic epitope-bearing

fragments present in most of the lung or lung cancer associated polynucleotides described in Table 1 as predicted by the inventors using the algorithm of Jameson and Wolf, (1988) Comp. Appl. Biosci. 4:181-186. The Jameson-Wolf antigenic analysis was performed using the computer program PROTEAN (Version 3.11 for the Power MacIntosh, DNASTAR, Inc.,  
5 1228 South Park Street Madison, WI). Lung and lung cancer associated polypeptides (e.g., SEQ ID NO:Y, polypeptides encoded by SEQ ID NO:X, or polypeptides encoded by the cDNA in the referenced cDNA clone) may possess one or more antigenic epitopes comprising residues described in Table 4. It will be appreciated that depending on the analytical criteria used to predict antigenic determinants, the exact address of the determinant  
10 may vary slightly. The residues and locations shown in column two of Table 4 correspond to the amino acid sequences for most lung and lung cancer associated polypeptide sequence shown in the Sequence Listing.

Table 5 shows the cDNA libraries sequenced, and ATCC designation numbers and vector information relating to these cDNA libraries.

15

### Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

20 In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original  
25 environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

30 As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X (as described in column 1 of Table 1) or the related cDNA clone (as described in column 9 of Table 1 and contained within a library deposited

with the ATCC). For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having an amino acid sequence encoded by a polynucleotide of the invention as broadly defined (obviously  
5 excluding poly-Phenylalanine or poly-Lysine peptide sequences which result from translation of a polyA tail of a sequence corresponding to a cDNA).

In the present invention, "SEQ ID NO:X" was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing  
10 all or most of the sequence for SEQ ID NO:X is deposited at Human Genome Sciences, Inc. (HGS) in a catalogued and archived library. As shown in column 9 of Table 1, each clone is identified by a cDNA Clone ID. Each Clone ID is unique to an individual clone and the Clone ID is all the information needed to retrieve a given clone from the HGS library. In addition to the individual cDNA clone deposits, most of the cDNA libraries from which the  
15 clones were derived were deposited at the American Type Culture Collection (hereinafter "ATCC"). Table 5 provides a list of the deposited cDNA libraries. One can use the Clone ID to determine the library source by reference to Tables 2 and 5. Table 5 lists the deposited cDNA libraries by name and links each library to an ATCC Deposit. Library names contain four characters, for example, "HTWE." The name of a cDNA clone ("Clone ID") isolated  
20 from that library begins with the same four characters, for example "HTWEP07". As mentioned below, Table 1 correlates the Clone ID names with SEQ ID NOs. Thus, starting with a SEQ ID NO, one can use Tables 1, 2 and 5 to determine the corresponding Clone ID, from which library it came and in which ATCC deposit the library is contained. Furthermore, it is possible to retrieve a given cDNA clone from the source library by techniques known in  
25 the art and described elsewhere herein. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides  
30 capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, or the complement thereof (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein), and/or sequences contained in the

related cDNA clone within a library deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also included within "polynucleotides" of the present invention are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA<sup>+</sup> sequences (such as any 3' terminal polyA<sup>+</sup> tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single-

and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both  
5 RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

10 In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a  
15 portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

20 "SEQ ID NO:X" refers to a lung cancer antigen polynucleotide sequence described in Table 1. SEQ ID NO:X is identified by an integer specified in column 1 of Table 1. The polypeptide sequence SEQ ID NO:Y is a translated open reading frame (ORF) encoded by polynucleotide SEQ ID NO:X. There are 443 lung cancer antigen polynucleotide sequences described in Table 1 and shown in the sequence listing (SEQ ID NO:1 through SEQ ID  
25 NO:443). Likewise there are 443 polypeptide sequences shown in the sequence listing, one polypeptide sequence for each of the polynucleotide sequences (SEQ ID NO:444 through SEQ ID NO:886). The polynucleotide sequences are shown in the sequence listing immediately followed by all of the polypeptide sequences. Thus, a polypeptide sequence corresponding to polynucleotide sequence SEQ ID NO:1 is the first polypeptide sequence  
30 shown in the sequence listing. The second polypeptide sequence corresponds to the polynucleotide sequence shown as SEQ ID NO:2, and so on. In otherwords, since there are 443 polynucleotide sequences, for any polynucleotide sequence SEQ ID NO:X, a

corresponding polypeptide SEQ ID NO:Y can be determined by the formula  $X + 443 = Y$ . In addition, any of the unique "Sequence/Contig ID" defined in column 2 of Table 1, can be linked to the corresponding polypeptide SEQ ID NO:Y by reference to Table 4.

The polypeptides of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

The lung and lung cancer polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides,



recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

5 The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

10 The lung and lung cancer polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant  
15 sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the polypeptides of the present invention in methods which are well known in the art.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length  
20 (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

25 "A polypeptide having functional activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular assay, such as, for example, a biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-  
30 dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less

and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

The functional activity of the lung cancer antigen polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

5 For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the present invention for binding to an antibody to the full length polypeptide antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich"  
10 immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one  
15 embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

20 In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment,  
25 physiological correlates polypeptide of the present invention binding to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and fragments, variants derivatives and analogs thereof to elicit polypeptide related biological  
30 activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

**Lung and Lung Cancer Associated Polynucleotides and Polypeptides of the Invention**

It has been discovered herein that the polynucleotides described in Table 1 are expressed at significantly enhanced levels in human lung and/or lung cancer tissues.

5 Accordingly, such polynucleotides, polypeptides encoded by such polynucleotides, and antibodies specific for such polypeptides find use in the prediction, diagnosis, prevention and treatment of lung related disorders, including lung cancer as more fully described below.

Table 1 summarizes some of the polynucleotides encompassed by the invention (including contig sequences (SEQ ID NO:X) and the related cDNA clones) and further  
10 summarizes certain characteristics of these lung and/or lung cancer associated polynucleotides and the polypeptides encoded thereby.

Table 1

Sequence/  
Seq ID No. Contig ID

Gene Name		Overlap	HGS Nucleotide		% Identity	% Similarity	Clone ID
Seq ID No.	Contig ID		Start	End			
1	507002	nuclear protein Skip [Homo sapiens] >gi3417599 (AF045184) nuclear receptor coactivator NCoA-62 [Homo sapiens]	2	970	91	91	HAJAW79
2	508935	(AF053651) cellular apoptosis susceptibility protein [Homo sapiens] >sp O75432 O75432 CELLULAR APOPTOSIS SUSCEPTIBILITY PROTEIN. Length = 971	1	1920	98	98	HDLAG79
3	518959	IgG Fc fragment receptor precursor [Homo sapiens] >pir JL0118 JL0118 Fc gamma (IgG) receptor IIa precursor - human >sp P12318 FCGA_HUMAN	257	727			HIBCM71
4	539756	LOW AFFINITY IMMUNOGLOBULIN GAMMA FC RECEPTOR II-A PRECURSOR (FC-GAMMA RI1-A) (FCRII-A) (IGG FC RECEPTOR II-A) (CD32)	51	293	98	98	HAPBV45
5	540125	cyclin H [Homo sapiens] >gi532561 cyclin H [Homo sapiens] >pir 38731 38731 cyclin H - human >sp P51946 CYCH_HUMAN CYCLIN H (MO15-ASSOCIATED PROTEIN) (P37) (P34). Length = 323	80	1099	95	95	HFPCA09
6	540275	leukotriene A-4 hydrolase precursor [Homo sapiens] >gi307131 leukotriene A4 hydrolase [Homo sapiens] >gi976396 leukotriene A4 hydrolase [Homo sapiens]	72	1946	100	100	HISBAG12

7	540331					235	519			HAPOO45
8	540955					1052	1492			HMAJ62
9	541251	M1 subunit of ribonucleotide reductase [Homo sapiens] >gil36153 large subunit ribonucleotide reductase [Homo sapiens] >pir[S16680]S16680 ribonucleoside-diphosphate reductase (EC 1.17.4.1) chain M1 - human Length = 792	gil36065			9	1469	98		HHFFY81
10	541978	collagen alpha 3(VI) chain precursor - human Length = 2970	pir[S13679]CGHU3A	2			991	99		HOHBK75
11	547680	actin bundling protein [Homo sapiens] >gil458028 actin bundling protein [Homo sapiens] >pir[38621]38621 actin bundling protein - human >sp[Q16638]FASC HUMAN FASCIN (ACTIN BUNDLING PROTEIN). Length = 493	gil497269	1			840	100		IIDP11109
12	547705	galactose-1-phosphate uridylyl transferase [Homo sapiens] >gil182951 galactose-1-phosphate uridylyl transferase [Homo sapiens]	gil182951	3			767	94		HCHMZ75
13	549763	cyclin-dependent protein kinase, type 4 [Homo sapiens] >gil1353416 cyclin-dependent kinase 4 [Homo sapiens] >sp[PI1802]CDK4_HUMAN CELL DIVISION PROTEIN KINASE 4 (EC 2.7.1.-) (CYCLIN-DEPENDENT KINASE 4) (PSK-13). Length = 303	gil456427	291			1124	92		HMGBP27
14	549819	suftactant apoprotein 18 precursor [Homo sapiens] Length = 243	gil338298	1			105	100		HAPQT93
15	549820	suftactant apoprotein 18 precursor [Homo sapiens] Length = 243	gil338298	3			464	75		HFTDY88

16	549944	von Willebrand factor [Homo sapiens] >pir A34480 VWHU von Willebrand factor precursor - human >gil553810 von Willebrand factor [Homo sapiens] (SUB 990-1947) >gnl PIDe222518 von Willebrand factor [Homo sapiens] (SUB 1-178) >gil340316 von Willebrand antige	gil340356	741	1928	100	100	HUVEG60
17	551426	connexin 40 [Homo sapiens] Length = 358	gil1220303	1	372	97	97	HAPQN48
18	552182	macrophage lectin 2 [Homo sapiens] >sp Q14538 Q14538 MACROPHAGE LECTIN 2. Length = 292	gnl PID d1009736	696	923	98	98	HDPFQ65
19	552540	lung surfactant protein D [Homo sapiens] Length = 375	gil34767	1	414	96	96	HAPQD13
20	553367	(AF053944) aortic carboxypeptidase-like protein ACLP [Homo sapiens] >sp G328891 G3288916 AORTIC CARBOXYPEPTIDASE-LIKE PROTEIN ACLP. >gnl PID d1013781 AEBP1 [Homo sapiens] (SUB 314-1158) Length = 1158	gil3288916	423	1160	98	99	HD1DB06
21	554326	SAS [Homo sapiens] >sp O00577 O00577 COSMID 6E5 CDK4, SAS AND KIAA0167 GENES, COMPLETE CDS, AND OS9. Length = 227	gil2209293	500	1195	92	93	HSDJF42
22	554657	biliverdin-IXbeta reductase 1 [Homo sapiens] >gnl PID d1005912 NADPH-flavin reductase [Homo sapiens] >pir JC2070 JC2070 NADPH dehydrogenase (flavin) (EC 1.6.8.2) - human >sp P30043 FLRE HUMAN FLAVIN REDUCTASE (EC 1.6.99.1) (FR) (NADPH- DEPENDENT DIAPHORASE	gnl PID d1007449	69	713	93	93	HIMUAP34

23	556156	nuclear chloride ion channel protein [Homo sapiens] >sp O0299 CL11_HUMAN CHLORIDE INTRACELLULAR CHANNEL PROTEIN 1 (NUCLEAR CHLORIDE ION CHANNEL 27) (P64 CLCP). Length = 241	gi 2073569	243	635	94	96	HMEFP33
24	557747	prepro-plasma carboxypeptidase B [Homo sapiens] >pir A41204 A41204 carboxypeptidase B (EC 3.4.17.2) CPB2 precursor - human >sp Q15114 Q15114 PREPRO-PLASMA CARBOXYPEPTIDASE B. Length = 423	gi 189687	113	745	96	96	HLQBO43
25	558599	COX5B [Homo sapiens] >gi 180941 cytochrome c oxidase precursor (EC 1.9.3.1) [Homo sapiens] >pir T0324 OTHUSB cytochrome-c oxidase (EC 1.9.3.1) chain Vb precursor - human	gi 180937	2	478	87	88	HMCBO59
26	572403	VDUP1=1,25-dihydroxyvitamin D-3 up-regulated [human, HL-60 promyelocytic leukemia cells, Peptide, 391 aa] [Homo sapiens] Length = 391	bb 155932	434	607	94	94	IIIJDI116
27	573366			41	163			HBAMD71
28	573986			159	338			HYACJ68
29	575435			297	1490			IIIIIM157
30	584341	pulmonary surfactant-associated protein [Homo sapiens] Length = 248	gi 190672	90	866	99	99	HAPOA63
31	584435	CD53 glycoprotein [Homo sapiens] >gi 180141 cell surface antigen [Homo sapiens] >pir A37243 A37243 hemopoietic cell surface glycoprotein CD53 - human >sp P19397 CD53_HUMAN LEUKOCYTE SURFACE ANTIGEN CD53 (CELL SURFACE GLYCOPROTEIN CD53). Length = 219	gi 180143	122	814	89	89	HUFAR85

32	585187	enigma protein [Homo sapiens] >pir[A.55050]A.55050 enigma - human	gij1561637	1	1494	77	79	HMEJD03
33	585658	transcobalamin II [Homo sapiens] >pir[A.39744]A.39744 transcobalamin II precursor - human >gij2952291 (AF047576) transcobalamin II [Homo sapiens] (SUB 1-21) >sp[G298394]G298394 TRANSCOBALAMIN II ISOPEPTIDE A (N- TERMINAL). (SUB 1-26) Length = 427	gij339196	85	888	91	91	HMACGB31
34	585693	PECAM-1 precursor [Homo sapiens] >pir[A.40096]A.40096 platelet-endothelial cell adhesion molecule-1 (CD31) precursor - human >sp[P16284]PEC1_HUMAN PLATELET ENDOTHELIAL CELL ADHESION MOLECULE PRECURSOR (PECAM-1) (CD31 ANTIGEN) (ENDOCAM) (GPIIA'). >bbs13897	gij189776	759	2405	92	92	HMSAO07
35	585701	preprocathepsin H (AA -22 to 314) [Homo sapiens] >pir[S12486]KHHUH cathepsin H (EC 3.4.22.16) precursor - human >sp[P09668]CATH_HUMAN CATHEPSIN H PRECURSOR (EC 3.4.22.16). >gij29708 cathepsin H [Homo sapiens] (SUB 88- 335) Length = 335	gij29710	3	1094	99	100	HDPJP49
36	586019	major group rhinovirus receptor precursor [Homo sapiens] Length = 532	gij306895	2	1354	95	95	HOGAH59
37	587225	cytotoxin serine protease-C precursor [Homo sapiens] >gij183155 cytotoxic T-lymphocyte- associated serine esterase 1 [Homo sapiens] >gij181157 cytotoxic serine proteinase [Homo sapiens] >gij338430 serine protease [Homo sapiens] >pir[A.32692]A.32692 cytotoxic	gij181164	32	865	100	100	HWAAAB59



38	587445	Clara cells 10 kDa secretory protein [Homo sapiens] >gi 457933 Clara cells 10 kDa secretory protein [Homo sapiens] >gi 23132 10 kDa secretory preprotein (AA -21 to -1) [Homo sapiens] >pir JS0036 JS0036 Clara cell 10K protein precursor - human >pir 138397]	gi 457933	75	431	91	93	HLJBE03
39	587572	pulmonary surfactant protein SP-C1 [Homo sapiens] Length = 191	gi 387030	30	677	91	91	HTFB376
40	587596	acid phosphatase type 5 (AA 1 - 325) [Homo sapiens] >pir S15752 S15752 acid phosphatase (EC 3.1.3.2) ACP5 precursor - human		20	190			HAIQS32
41	588548	>sp G262924 G262924 TARTRATE-RESISTANT ACID PHOSPHATASE PEAK 1 ISOFORM 16 KDA SUBUNIT, TRACP PEAK 1 (N- TERMINAL). {SUB 183-203} Len	gi 34734	97	1092	95	95	HAPNX70
42	588881	monocyte antigen CD14 [Homo sapiens] >gi 29741 leucine-rich preprotein (AA -19 to 356) [Homo sapiens] Length = 375	gi 180021	240	1532	89	89	HHFCG79
43	588933	heparin cofactor II [Homo sapiens] >pir A37924 A37924 heparin cofactor II precursor - human >sp P05546 HEP2_HUMAN HEPARIN COFACTOR II PRECURSOR (HC-II) (PROTEASE INHIBITOR LEUSERPIN 2) (HLS2). >gi 412013 proteinase inhibitor [unidentified] {SUB 20-499} >g	gi 183908	3	1532	97	97	HFVHH90
44	592136	cytochrome P450 [Homo sapiens] >pir A54116 A54116 cytochrome P450 1B1 - human >sp Q16678 CYP1B_HUMAN CYTOCHROME P450 1B1 (EC 1.14.14.1) (CYP1B1). Length = 543	gi 501031	422	991	88	89	HDPGB64

[illegible]

50	625480	CD68=110kda transmembrane glycoprotein [human, promonocyte cell line U937, Peptide, 354 aa] [Homo sapiens] >pir A48931 A48931 transmembrane glycoprotein CD68, 110K - human >sp P34810 CD68_HUMAN MACROSIALIN PRECURSOR (GP110) (CD68). >gi 3511124 (AF060540)	bsl 127493	1	1074	65	65	HDPXE17
51	647688	ALDH7 [Homo sapiens] >pir I38669 I38669 ALDH7 - human >sp P43353 DHA7_HUMAN ALDEHYDE DEHYDROGENASE 7 (EC 1.2.1.5). >sp G601780 G601780 ALDH7. Length = 468	gi 601780	1	1290	85	88	HCHCC79
52	650865	DOC1 [Homo sapiens] >sp Q13597 Q13597 MYOSIN HEAVY CHAIN HOMOLOG DOC1. Length = 752	gi 1297319	251	1567	99	99	HMSBY41
53	651676	retinoic acid binding protein II [Homo sapiens] >gi 181030 retinoic acid-binding protein II [Homo sapiens] >pir A45057 RJHU2 retinoic acid-binding protein II, cellular - human Length = 138	gi 181026	120	566	100	100	HMAID66
54	651751	ADH beta-1-subunit (aa 1-375) [Homo sapiens] >gi 178111 alcohol dehydrogenase beta-1 subunit [Homo sapiens] >gnl PID d1000528 alcohol dehydrogenase beta 1 [Homo sapiens] >pir A23607 DEHUAB alcohol dehydrogenase (EC 1.1.1.1) 2 - human >sp P00325 ADHB_HUMAN	gi 28416	3	1187	99	99	HLD0U10

55	651787	hla-dr antigen alpha chain [Homo sapiens] >gi 386945 HLA-DR alpha-chain [Homo sapiens] >gi 307267 HLA-DR alpha-chain [Homo sapiens] >pir A93952 HLHUDA MHC class II histocompatibility antigen HLA-DR alpha chain precursor - human >sp P01903 HA2R_HUMAN HLA C	gi 307264	2	844	91	91	HDPMQ14
56	651840	creatine kinase [Homo sapiens] >gi 3702298 (AC005781) KCRM_HUMAN; M-CK [Homo sapiens] >pir A31793 KIHUCM creatine kinase (EC 2.7.3.2) chain M - human >sp P06732 KCRM_HUMAN CREATINE KINASE, M CHAIN (EC 2.7.3.2) (M-CK). >sp G3702298 G3702298 KCRM_HUMAN (EC	gi 180588	2	1339	96	96	HHBHB11
57	651892	TGF-b superfamily receptor type I [Homo sapiens] >gi 2228562 activin receptor like kinase 1 [Homo sapiens] >pir A49431 A49431 TGF-b superfamily receptor type I - human >sp P37023 KIR3_HUMAN SERINE/THREONINE-PROTEIN KINASE RECEPTOR R3 PRECURSOR (EC 2.7.1.3	gi 425148	994	1587	93	96	HEMDR71
58	652557	GLI-Krupple related protein [Homo sapiens] >pir A40350 A40350 transcription repressor protein YY1 - human Length = 414	gi 186768	3	1064	92	92	HBMVZ88
59	653011	(AF068836) cytohesin binding protein HE [Homo sapiens] >sp O60759 O60759 CYTOHESIN BINDING PROTEIN HE. Length = 359	gi 3192909	409	651	93	93	HRADN49
60	656155			57	233			HLIAR11
61	656930			88	840			HTXFS01

62	659023	alpha-1 type IV collagen [Homo sapiens] >pir S16876 CGHU4B collagen alpha 1(IV) chain precursor - human >sp P02462 CA14_HUMAN PROCOLLAGEN ALPHA 1(IV) CHAIN PRECURSOR. >gil 180424 pro-alpha-1(IV) [Homo sapiens] (SUB 1256-1669) >gil 180818 procollagen alpha-1	gil 180803	2	337	HRDBI73 HSICM51 HSLFT56
63	659263			98	412	
64	660696			1	1218	
65	666881	(AC002073) Lim Kinase [Homo sapiens] >gnl PID d1008908 LIMK-2 [Homo sapiens] >sp P53671 LIK2_HUMAN LIM DOMAIN KINASE 2 (EC 2.7.1.-) (LIMK-2). >pir PC4291 P4291 LIMK2a protein - human (fragment) (SUB 1-80) Length = 638	gil 2078472	101	373	HOIECM70
66	677071	hepatoma-derived GF [Homo sapiens] >pir A55055 A55055 hepatoma-derived growth factor - human >sp P51858 HDGF_HUMAN HEPATOMA-DERIVED GROWTH FACTOR (HDGF). Length = 240	gnl PID d1004419	492	713	HODDC39
67	677997			23	163	HAPMF55
68	681507			1	738	HOEFV86
69	682736	selenophosphate synthetase 2 [Homo sapiens] >sp Q99611 Q99611 SELENOPHOSPHATE SYNTHETASE 2. Length = 448	gil 1815622	109	858	HGBASI1
70	683116	rsec8 [Rattus norvegicus] >pir J59422 J59422 rsec8 - rat (fragment) >sp Q62824 Q62824 RSEC8 (FRAGMENT). Length = 975	gil 1019441	93	1346	HMSCX18
71	686494			797	979	HK1YK88

72	686634	HLA DP4 beta-chain [Homo sapiens] >gi 296648 pot. hla-dp-beta 1 [Homo sapiens] >pir A02229 HLHUPB MHC class II histocompatibility antigen III.A-DP beta 1 chain (allele DPB4.1) precursor - human >sp P04440 HB2P_HUMAN HLA CLASS II HISTOCOMPATIBILITY ANTIGEN.	gi 306858	325	867	97	97	HABBM85
73	688221	(AF033095) testis enhanced gene transcript protein [Homo sapiens] Length = 237	gi 2645729	60	824	78	78	HCYBK57
74	703498	MacMARCKS gene product [Homo sapiens] >pir S31861 S31861 myristylated alanine-rich protein kinase C substrate, macrophage - human >sp P49006 MRP_HUMAN MARCKS-RELATED PROTEIN (MAC-MARCKS). {SUB 2-195} Length = 195	gi 38435	27	767	67	67	HINTSA18
75	705143	collagen alpha 2(IV) chain precursor - human >sp P08572 CA24_HUMAN PROCOLLAGEN ALPHA 2(IV) CHAIN PRECURSOR. >gi 29551 alpha (2) chain [Homo sapiens] {SUB 1254-1712} >gi 553233 alpha-2 type IV collagen [Homo sapiens] {SUB 1-33} Length = 1712	pir A32024 CGHU2B	1056	2345	100	100	HLDCTU68
76	705227			1185	1388			HABQ62
77	705958	pulmonary surfactant-associated protein SP-B [Homo sapiens] Length = 381	gi 190674	1	291	97	97	HAPSA56
78	705965	proteasome subunit LMP7 [Homo sapiens] >gi 1054747 alternative first exon (1b) [Homo sapiens] >pir C4324 C44324 proteasome chain LMP7, form E2 precursor - human Length = 276	gi 38482	371	727	99	99	HHFGD34
79	706145			2	787			HIELFV83

80	706473			605	934		HT3BL17
81	707380			2	160		HOSC174
82	707779	ubiquitin-conjugating enzyme [Homo sapiens] >gnlP1Dle228278 ubiquitin conjugating enzyme [Homo sapiens] >gij1184055 ubiquitin conjugating enzyme homolog [Homo sapiens] >gij1574950 RAD6 homolog: May be involved in ubiquitin conjugation; Interacts with RAD	gij1172224	2	616	100	HOUGS29
83	709441	c-syn protooncogene [Homo sapiens] >pirA24314TVHUSY protein-tyrosine kinase (EC 2.7.1.112) fyn, splice form B - human >spj06241fYN_HUMAN PROTO-ONCOGENE TYROSINE-PROTEIN KINASE FYN (EC 2.7.1.112) (P59-FYN) (SYN) (SLK). (SUB 2-537) Length = 537	gij1181172	3	272	96	HIFCC93
84	710443	adenylyl cyclase-associated protein [Homo sapiens] >gij179920 CAP protein [Homo sapiens] >pirA48120/A48120 adenylyl cyclase-associated CAP protein homolog - human >spjQ01518CAP1_HUMAN ADENYLYL CYCLASE-ASSOCIATED PROTEIN 1 (CAP 1). Length = 475	gij178084	124	1560	100	HUSAK67
85	710603	tissue-specific secretory protein [unidentified] >gij818881 epididymal secretory protein precursor [Pan troglodytes] >gij794071 epididymal secretory protein 14.6 [Macaca fascicularis] >gij37477 orf [Homo sapiens] >pirj53929j53929 epididymal secretory pr	gij513467	285	2099	90	HCLCA40
86	710616			13	549	90	HLJBI80





102	723968	cytochrome c oxidase subunit VIb (AA 1-86) [Homo sapiens] >gi 30381 cytochrome oxidase subunit VIb [Homo sapiens] >gi 2098574 (AC002115) COXG [Homo sapiens] >pir S03287 OGHU6B cytochrome-c oxidase (EC 1.9.3.1) chain VIb - human >sp P14854 COXG_HUMAN CYTOC	gi 30295	64	408	100	100	HLDXB30
103	725321			2	337			HUKER20
104	725326	DNA-binding protein [Homo sapiens] >sp E33113 E331113 DNA-BINDING PROTEIN. >sp G2275153 G2275153 DNA-BINDING PROTEIN. Length = 426	gi 2275153	3	665	94	94	HTXKL35
105	726034	FIBRONECTIN PRECURSOR (FN). >gi 182697 fibronectin [Homo sapiens] {SUB 1594-2386} >gi 4096852 fibronectin [Homo sapiens] {SUB 1116-1422} >gi 4096850 fibronectin [Homo sapiens] {SUB 2228-2386} >gi 4096858 fibronectin [Homo sapiens] {SUB 2231-2386} >gi 1826	sp P02751 FINC_HUMAN	175	867	98	98	HSLBF52
106	726602			2	247			HAPNN47
107	726965			882	1220			HBKDL66
108	727809	Na,K-ATPase beta subunit [Homo sapiens] >gi 28933 pu. Na/K-ATPase beta (aa 1-303) [Homo sapiens] >pir A23764 PWHUNB Na+/K+-exchanging ATPase (EC 3.6.1.37) beta chain - human >sp P03026 ATNB_HUMAN SODIUM/POTASSIUM-TRANSPORTING ATPASE BETA-1 CHAIN (EC 3.6.	gi 386974	103	1017	95	95	HEBGA63
109	731703			106	423			HTTEM33

110	732840	(AB019219) similar to yeast pre-mRNA splicing factors, Prp1/Zer1 and Prp6 [Homo sapiens] Length = 941	gnl p D d 1038129	752	1456	100	100	HEONN38
111	733629	hevin gene product [Homo sapiens]		320	502			HEKAL56
112	733749	>pir S60062 S60062 hevin precursor - human >sp Q14515 Q14515 HIGH ENDOTHELIAL VENULE PRECURSOR. Length = 664	gij 758066	221	2272	86	86	HNTN108
113	734119			1440	1622			HWADN83
114	734637	transfer RNA-Trp synthetase [Homo sapiens] >pir JH0533 JH0533 tryptophan--tRNA ligase (EC 6.1.1.2) - human Length = 471	gij 340368	324	749	93	95	HTXEJ03
115	734638	transfer RNA-Trp synthetase [Homo sapiens] >gij 30821471 aa polypeptide (gamma2) [Homo sapiens] >pir A41633 A41706 tryptophan--tRNA ligase (EC 6.1.1.2) - human >bbs 179357 tryptophanyl-tRNA synthetase, TrpRS [N-terminal, alternatively spliced] (EC 6.1.1.2)	gij 184657	511	1935	100	100	HMEJM56
116	734865	CAG-isl 7 [Homo sapiens] Length = 213	gij 3126984	1	795	89	89	HEGAJ73
117	738846			234	356			HAPOQ59
118	740584			103	246			HBMUW84
119	741213	(AF071559) histone deacetylase dHDAC3 [Drosophila melanogaster] >sp G3982757 G3982757 HISTONE DEACETYLASE HDAC3. Length = 438	gij 3982757	3	506	43	72	HAPNP64

120	741229	unnamed protein product [Homo sapiens] >gi903934 cysteine protease [Homo sapiens] >gi886050 [ch-2 [Homo sapiens] >gi999454 TX protease precursor [Homo sapiens] >gi4096346 Mlh1/TFX isoform alpha [Homo sapiens] >pirA57511/A57511 interleukin-1 beta contig	gnl PI D e306342	60	1115	94	94	HIWLEGG61
121	741299	(AJ010952) putative tRNA splicing protein [Homo sapiens] >sp E1321525 E1321525 PUTATIVE TRNA SPLICING PROTEIN (FRAGMENT). Length = 318	gnl PI D e1321525	2	955	100	100	HOEEY69
122	743134	P47 LBC oncogene [Homo sapiens] >pir 38434 38434 P47 LBC oncogene - human >sp Q12802 Q12802 P47 LBC ONCOGENE. Length = 424	gi 458210	1	1254	99	99	HAMGO15
123	744680	IGF-BP 4 [Homo sapiens] >gnl PI D e1227579 insulin-like growth factor binding protein 4 [Homo sapiens] >pir B37252 B37252 insulin-like growth factor-binding protein 4 precursor - human >sp P22692 BP4_HUMAN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 4 PREC	gi 184816	379	747	99	99	HUSGU74
124	744705	protein phosphatase 2A 65 kDa regulatory subunit, alpha isoform [Sus scrofa] >sp P54612 2AAA_PIG	gi 510469	69	983	99	100	HPMGT42
125	745337	PROTEIN PHOSPHATASE PP2A, 65 KD REGULATORY SUBUNIT, ALPHA ISOFORM (PROTEIN PHOSPHATASE PP2A SUBUNIT A, ALPHA ISOFORM) (PR65-ALPHA). (SUB 2-589) Length = 589		50	1837			HADDU62

126	745570					1044	1322		HMQB61
127	746078					25	201		HAGV41
128	750595					666	1061		HE8PW74
129	750633					768	1202		HAUDK02
130	750766					1	858	90	HE8PS92
131	752225					238	426		HLWFK49
132	754538					1	567		HAFST69
133	754820					103	561	100	HLDAR05
134	756565					78	221	90	HOSCN57
135	756793								
136	757431					1603	1896		IICRA131
						145	726	100	HSA YH89
137	757478								
138	757695					3	578		HTWDN75
139	760876					636	971		HISAG86
140	761528					1	240		HCLCO72
						2	1273	96	HSICU72
141	761936								
						454	645		HAFBF11

CLE7 [Gallus gallus] Length = 239

apolipoprotein E [Homo sapiens] Length = 317

GTP-binding protein (rnb5) [Canis familiaris]  
>pir|A30413|A30413 GTP-binding protein Rab5 -  
dog Length = 215(AF021819) RNA-binding protein regulatory subunit  
[Homo sapiens] >sp|O14805|O14805 RNA-  
BINDING PROTEIN REGULATORY SUBUNIT.  
Length = 189fructose 1,6-bisphosphatase (EC 3.1.3.11) [Homo  
sapiens] >gnl|P1D|d1005592 'fructose-1,6-  
bisphosphatase' [Homo sapiens] >gnl|P1D|d1005593  
'fructose-1,6-bisphosphatase' [Homo sapiens]  
>gnl|P1D|d1005594 'fructose-1,6-bisphosphatase'  
[Homo sapiens] >sp

142	761944	homologue of yeast IPP isomerase [Homo sapiens] >pir A53028 A53028 isopentenyl-diphosphate Delta- isomerase (EC 5.3.3.2) homolog - human >sp Q13907 Q13907 HOMOLOG OF YEAST IPP ISOMERASE. Length = 228	gi 488750	1342	1923	94	94	H6FEL27
143	764913	keratinocyte growth factor 2 [Homo sapiens] >gnl P D d1023194 (AB002097) FGF-10 [Homo sapiens] >sp O15520 O15520 FIBROBLAST GROWTH FACTOR-10. Length = 208	gi 2231295	486	1214	89	89	HOEFN72
144	764941	ras inhibitor [Homo sapiens] >pir C38637 C38637 Ras inhibitor (clone JC310) - human (fragment) >sp Q00426 Q00426 PUTATIVE RAS INHIBITOR (FRAGMENT). Length = 428	gi 553634	672	1769	97	99	HDPQI77
145	765903	Sec62 [Homo sapiens] >gnl P D d1013944 translocation protein-1 [Homo sapiens] >pir C5279 C5279 translocation protein 1 - human >sp Q99442 Q99442 TRANSLOCATIONAL PROTEIN-1. Length = 399	gi 1928973	766	1092	100	100	IKOSC281
146	766122			3	224			HAPST63
147	766719			448	822			HFASM02
148	767655			86	1162			HCUCCT6
149	767941	HPBR11-7 gene product [Homo sapiens] >gi 871299 Human pre-mRNA cleavage factor 1 68 kDa subunit [Homo sapiens] >pir S57447 S57447 HPBR11-7 protein - human >sp Q16630 Q16630 HPBR11-4 MRNA. Length = 551	gi 871301	2	334	68	85	HTGDY23
150	768035	5C5. Length = 276	sp Q15693 Q15693	170	655	82	82	HHFCX49
151	769888			106	690			HGBCO73

152	771671	complement C1q B-chain precursor [Homo sapiens] >pijB23422C1HUQB complement subcomponent C1q chain B precursor - human {SUB 3-255} >spIP02746C1QB_HUMAN COMPLEMENT C1Q SUBCOMPONENT, B CHAIN PRECURSOR. {SUB 5-255} >gij573114 C1q B-chain precursor [Homo s	gij179642	594	890	98	100	HDPK07
153	772876	dj262D12.2 ((mitochondrial/chloroplast 30S ribosomal protein S14)-LIKE protein) [Homo sapiens] >spO60783jO60783 DJ262D12.2 (MITOCHONDRIAL/CHLOROPLAST 30S RIBOSOMAL PROTEIN S14)-LIKE PROTEIN). Length = 128	gnljPIDje1284377	59 145	820 489	99	99	HUSIR49 HDLAB47
155	773398	(AF089816) RGS-GAIP interacting protein GIPC [Homo sapiens] >spG3789934G3789934 RGS- GAIP INTERACTING PROTEIN GIPC. >gij2613004 (AF028824) Tax interaction protein 2 [Homo sapiens] {SUB 78-333} Length = 333	gij3789934	699 501	995 953	90	90	HAFBD20 HWLFD70
156	773647							
157	773927	splicing factor [Homo sapiens] >gij472956 gClq-R [Homo sapiens] >pijJT0762JT0762 pre-mRNA splicing factor SF2 P32 chain precursor - human >spIQ0702IJMA32_HUMAN COMPLEMENT COMPONENT 1, Q SUBCOMPONENT BINDING PROTEIN PRECURSOR (GLYCOPROTEIN GC1QBP) (GC1Q-	gij338045	1	945	100	100	HCHME48

158	774100	(AF006088) p16-Arc [Homo sapiens] >gi2407611 (AF017807) Arp2/3 complex 16kDa subunit [Homo sapiens] >sp O15511 AR16_HUMAN ARP2/3 COMPLEX 16 KD SUBUNIT (P16-ARC). Length = 151	gi2282042	97	597	100	100	HGCNL45
159	774101			120	353			HMVCT79
160	774159			1	141			HKAELV15
161	774341			2	682			HMAFC46
162	774371			1291	1647			HHFHK22
163	777534	(AF006082) Arp2 [Homo sapiens] >sp O15142 ARP2_HUMAN ACTIN-LIKE PROTEIN 2. Length = 394	gi2282030	148	1365	96	96	HMTAE25
164	777623	(AF030162) inner mitochondrial membrane translocase Tim23 [Homo sapiens] >sp O14925 O14925 INNER MITOCHONDRIAL MEMBRANE TRANSLOCASE TIM23. Length = 209	gi2599129	98	760	86	86	HMELH12
165	779194			2	232			HODFE80
166	779387	SSR alpha subunit [Homo sapiens] >pir J38246 J38246 SSR alpha subunit - human Length = 286	gi551638	138	1028	84	84	HNTNA20
167	779790			3	488			HMIAE54
168	779818	human complement C1r [Homo sapiens] >pir A24170 C1HURB complement subcomponent C1r (EC 3.4.21.41) precursor - human >sp P00736 C1R_HUMAN COMPLEMENT C1R COMPONENT PRECURSOR (EC 3.4.21.41). Length = 705	gi179644	73	1125	98	99	HLICR27

169	779819	human complement C1r [Homo sapiens] >pir A24170 CIHURB complement subcomponent C1r (EC 3.4.21.41) precursor - human >sp P00736 C1R_HUMAN COMPLEMENT C1R COMPONENT PRECURSOR (EC 3.4.21.41). Length = 705	gij 79644	370	1716	99	99	HIBEI12
170	780634	Man9-mannosidase [Homo sapiens] >pir S38965 S38965 Man(9)-mannosidase - human >sp P33908 MA12_HUMAN MANNOSYL-OLIGOSACCHARIDE ALPHA-1,2-MANNOSIDASE (EC 3.2.1.113) (MAN(9)-ALPHA-MANNOSIDASE). Length = 625	gij 416180	3	281	97	97	HLTGU89
171	780638	(AF049659) geranylgeranyl pyrophosphate synthase [Drosophila melanogaster] >sp O61539 O61539 GERANYLGERANYL PYROPHOSPHATE SYNTHASE. Length = 338	gij 2944400	209	1120	58	76	HDQER43
172	780773	(AC004079) 40% similar to yeast high mobility group-like nuclear protein, P32495 (PID:g417360) [Homo sapiens] >sp O43362 O43362 SIMILAR TO YEAST HIGH MOBILITY GROUP-LIKE NUCLEAR PROTEIN. Length = 151	gij 2822179	43	555	91	94	HBJHV91
173	780778	transcription factor LBP1a - human Length = 504	pir A56205 A56205	3	167	100	100	HE8TZ19
174	780873	DAD1 protein [Sus scrofa] >sp Q29036 DAD1_PIG-DEFENDER AGAINST CELL DEATH 1 (DAD-1). Length = 113	gnl PID d1013802	1	429	87	87	HEDAE01



175	782113	(AF039704) lysosomal pepstatin insensitive protease [Homo sapiens] >sp G406384 G406384.1 LYSOSOMAL PEPSTATIN INSENSITIVE PROTEASE. Length = 563	gij406384.1	575	1702	92	92	HWDEE06
176	782153	Mvp1p [Saccharomyces cerevisiae] Length = 511	gij56212.1	133	855	39	72	HDABR21
177	782376	similar to ubiquitin conjugating enzyme [Caenorhabditis elegans] >sp Q18931 Q18931 SIMILAR TO UBIQUITIN CONJUGATING ENZYME. Length = 309	gij746510	100	1014	62	79	HFXBY84
178	782420	leptin receptor gene-related protein [Homo sapiens] >sp O15243 O15243 OB-R GENE RELATED PROTEIN. Length = 131	gnl P1D e315497	1	480	100	100	HSKHA38
179	782672	(AJ000644) SPOP [Homo sapiens]		3	380			HOSAW38
180	783148			3	326			HBIBU44
181	783510	>sp O43791 O43791 SPOP. Length = 374	gnl P1D e1216712	165	1319	95	95	HCGBC59
182	783734	mena protein [Mus musculus] >sp P70430 P70430 ENABLED HOMOLOG (MENA PROTEIN). Length = 541	gij1644455	2	1171	81	84	HOHAH70
183	784201	(AF073839) bithoraxoid-like protein [Rattus norvegicus] >sp O88567 O88567 BITHORAXOID- LIKE PROTEIN. Length = 96	gij328888.1	64	477	93	96	HWHGB85
184	784381	tetracycline transporter-like protein [Mus musculus] >pir JC5641 JC5641 sugar transporter protein HIA T1 - mouse >sp P70187 P70187 HIPPOCAMPUS ABUNDANT PROTEIN TRANSCRIPT 1 (TETRACYCLINE TRANSPORTER-LIKE PROTEIN). Length = 490	dbj D88315_1	3	1409	78	84	HDQFG33
185	784387			2	472			HWBEJ50

186	784639	GTP-binding protein (rab7) [Canis familiaris] >pir B30413 B30413 GTP-binding protein rab7 - dog Length = 207	88	711	100	100	HE9RW49
187	784641	dJ560B9.3 [Homo sapiens] >sp O43735 O43735 DJ560B9.3. Length = 152	120	596	67	81	HDQEW56
188	785053	DOC-2 [Homo sapiens] >pir G02228 G02228 DOC-2 - human >sp P98082 DOC2_HUMAN DIFFERENTIALLY EXPRESSED PROTEIN 2 (DOC-2). >gil 110539 mitogen-responsive phosphoprotein [Homo sapiens] (SUB 1-229) Length = 770	586 2	729 2038	90	90	HMSFN30 HOEBZ31
190	785384	ERF-1 gene product [Homo sapiens] >pir S34854 S34854 epidermal growth factor-response factor 1 - human >gil 972116 ERF-1 protein [Sus scrofa] (SUB 299-337) Length = 338	18 12	524 1178	75	75	HT5FS84 HWAAY25
192	786283	(AB016068) Hs Sic24p [Homo sapiens] >sp O75844 O75844 HS STE24P. Length = 475	1	1020	88	88	1101FMB15
193	786335	HN1 [Mus musculus] >sp P97825 P97825 HEMATOLOGICAL AND NEUROLOGICAL EXPRESSED SEQUENCE 1 (HN1) (HN1). Length = 154	1	600	73	75	HTLGW81
194	786511	possesses similarity with C3HC4 type zinc finger domains [Caenorhabditis elegans] >sp Q17573 Q17573 C01G6.4 PROTEIN. Length = 170	1 69	162 689	67	86	HOSDD78 HCWUG30
195	787330						

196	787377	steroid receptor coactivator [Homo sapiens] >pir A57620 A57620 steroid receptor coactivator 1 - human >sp Q13420 Q13420 STEROID RECEPTOR COACTIVATOR. >pir PC4362 PC4362 steroid receptor coactivator-1 - human (fragment) {SUB 990-1061} Length = 1061	gil117915	1	426	81	81	HAPSS34
197	787662	(AB001993) glia maturation factor homologous protein [Homo sapiens] >gil3329382 (AF038956)		191	508			HLHAU33
198	788754	glia maturation factor beta [Homo sapiens]		143	2077			HNTAQ63
199	789351	>sp O60234 O60234 GLIA MATURATION FACTOR HOMOLOGOUS PROTEIN. Length = 142	unl PID d1026502	630	1070	99	99	1INI1B05
200	789466	(AF037206) RING zinc finger protein [Mus musculus] >sp O54966 O54966 RING ZINC FINGER PROTEIN. Length = 268		47	283			HAPNZ91
201	790396	protein-tyrosine phosphatase [Homo sapiens]		1	819			HSKXJ34
202	791673	>sp P28562 DUS1_HUMAN DUAL SPECIFICITY PROTEIN PHOSPHATASE 1 (EC 3.1.3.48) (EC 3.1.3.16) (MAP KINASE PHOSPHATASE-1) (MPK-1) (		343	606			HLTCB62
203	792080		gil2746337	360	659	97	100	HDQHN07
204	793025	>pir S29090 S29090 dual specificity phosphoprotein phosphatase (EC 3.1.3.-) 1 - human	gil29981	835	1404	96	96	HNFDI94

205	793043	(AF012872) phosphatidylinositol 4-kinase 230 [Homo sapiens] >sp G2326227 G2326227 PHOSPHATIDYLINOSITOL 4-KINASE 230. >gil598193 phosphatidylinositol 4-kinase [Homo sapiens] {SUB 1191-2044} Length = 2044	gil2326227	164	1183	99	99	HEOMG90
206	793386							
207	795144	elF3-p66 [Homo sapiens] >gil2351378 translation initiation factor elF3 p66 subunit [Homo sapiens] >sp O15371 O15371 EIF3-P66. Length = 548	gil2351378	1 2	642 1204		99	HKABJ75 HFOXO07
208	795911							
209	795962							
210	796221	glutathione-S-transferase homolog [Homo sapiens] >sp P78417 P78417 GLUTATHIONE-S-TRANSFERASE HOMOLOG. Length = 241	gil2393722	1323 145 57	1496 777 914	96	97	HOGAN95 HOHAR44 HMAAAC12
211	796283							
212	796392							
213	797655							
214	799486	anaphylatoxin C3a receptor [Homo sapiens] Length = 482	gil1511644	443 3 3 144	718 845 776 1598	95	95	HFTBV21 HEEAO23 HKMMC49 HTAFD81
215	799681							
216	800221	endothelial PAS domain protein 1 [Homo sapiens] Length = 870	gil1805268	41 1	217 999	98	98	HCLSC85 HHFPS31
217	800376	ABC-C transporter [Homo sapiens] >pir S71363 S71363 probable transport protein ABC-C - human >sp Q92473 Q92473 ABC-C TRANSPORTER. Length = 1704	gnl P1D e243436	3	557	100	100	HAP0J91
218	800567							
				1	1170			HTXDX21

219	800652			2	745		HMEFR61
220	800748	(AF075575) dysferlin [Homo sapiens] >sp O75923 O75923 DYSFERLIN. Length = 2080	gi 3600028	3	1850	83	HDQGA13
221	802032			153	560		HPJCA43
222	802050	glutathione peroxidase [Homo sapiens] >gnl PID d1000980 glutathione peroxidase [Homo sapiens] >sp O43787 O43787 GLUTATHIONE PEROXIDASE (EC 1.11.1.9). >gnl PID d1004380 plasma glutathione peroxidase [Homo sapiens] {SUB 82-226} >gnl PID d1004379 plasma glut	gnl PID e1192233	129	362	93	HAOMI:95
223	805551	(AF044201) neural membrane protein 35; NMP35 [Rattus norvegicus] >sp O88407 O88407 NEURAL MEMBRANE PROTEIN 35. Length = 316	gi 3426268	118	1056	61	HWHQN16
224	805662	atopy related autoantigen CALC [Homo sapiens] >sp O75785 O75785 ATOPY RELATED AUTOANTIGEN CALC (FRAGMENT). Length = 313	gnl PID e1310645	67	1527	99	HAITV32
225	805750			1	318		HE9QJ13
226	805860			3	317		HSSBJ90
227	805886	(AF090386) napsin A [Homo sapiens] Length = 420	gi 4154287	186	1040	99	HAPOP50
228	806706	endothelial PAS domain protein 1 [Homo sapiens] Length = 870	gi 1805268	179	649	91	HAHA^80

229	811637	(AF093414) estrogen response element binding protein [Saguinus oedipus] >sp O77798 O77798 ESTROGEN RESPONSE ELEMENT BINDING PROTEIN. >sp G386547 G386547 D(TTAGGG)N-BINDING PROTEIN B39=TYPE E HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN HOMOLOG (PEPTIDE 2). [SU]	gi 3747052	269	1189	88	88	H6EDL02
230	811782	(AF026124) schwannoma-associated protein [Mus musculus] >sp O35405 O35405 SCHWANNOMA-ASSOCIATED PROTEIN. Length = 488	gi 2565396	1012	1755	85	86	HUSHH56
231	812338	p40 [Homo sapiens] >sp O00368 O00368 RAB9 EFFECTOR P40. COMPLETE CDS. Length = 372	gnl PID e323546	261	1169	96	96	HAPTR06
232	812439	(AB008375) osteoblast specific cysteine-rich protein [Homo sapiens] >sp O14549 O14549 OSTEOBLAST SPECIFIC CYSTEINE-RICH PROTEIN. Length = 403	gnl PID d1023870	617	1699	85	85	HSLHL73
233	812645	GDP dissociation inhibitor [Homo sapiens] >gi 441455 Human rho GDP-dissociation Inhibitor 2(IEF 8120) [Homo sapiens] >pir A47742 A47742 Rho-GDP-dissociation inhibitor Ly-GDI - human >sp P52566 GDIS_HUMAN RHO GDP-DISSOCIATION INHIBITOR 2 (RHO GDI 2) (RHO-G	gi 404045	1	462	96	97	HAI'NO29
234	812770	tropomyosin, fibroblast - human >gi 37424 cytoskeletal tropomyosin (AA 1-248) [Homo sapiens] [SUB 1-239] Length = 248	pir A25530 A25530	26	835	79	79	IIWAAAY56

235	812893	mitogen inducible gene mig-2 [Homo sapiens] >pirS69890[S69890 mitogen inducible gene mig-2 - human >sp Q14840 Q14840 MITOGEN INDUCIBLE GENE MIG-2 (FRAGMENT). Length = 720	gi 505033	176	2311	58	81	HMAFL22
236	813080	(AF052433) katanin p80 subunit [Strongylocentrotus purpuratus] >sp O61585 O61585 KATANIN P80 SUBUNIT. Length = 690	gi 3005601	2	538	37	60	HAPOV32
237	813139	phosphoprotein p53 [Homo sapiens] >sp Q16811 Q16811 CELLULAR TUMOR ANTIGEN P53 (FRAGMENT). Length = 393	gi 386994	337	1149	100	100	HHEUB27
238	815326	The hsa1520 gene product is novel. [Homo sapiens] >gi 125240 lysosomal-associated multitransmembrane protein [Homo sapiens] (SUB 8-269) Length = 269	gn PID d1008225	1204	1320	91	91	HDPRN26
239	815740			1243	1494			HLTEI46
240	815812			852	1028			HLTBF42
241	824865			2	904			HDP1H94
242	825138	(AF035819) macrophage receptor MARCO [Homo sapiens] >sp G3002791 G3002791 MACROPHAGE RECEPTOR MARCO. Length = 520	gi 3002791	176	484	100	100	HWLLC18
243	825535			2	736			HELGU27
244	826203			1604	1903			HL4AF72
245	827046			2477	2656			HE8BI56
246	827168			87	1295			HMSJA80

247	827195	collagen alpha 2(VI) chain precursor, medium splice form - human >sp P12110 CA26_HUMAN COLLAGEN ALPHA 2(VI) CHAIN (FRAGMENT). (SUB 237-589) >gi 179710 alpha-2 collagen type VI-a [Homo sapiens] (SUB 590-917) >gi 30050 precursor polypeptide (AA -20 to 234)	pir S09646 S09646	3	1484	98	99	HSYCG31
248	827249	(AF093119) UPH1 [Homo sapiens] >sp O75967 O75967 UPH1. Length = 439	gi 3676824	178	1476	98	98	HSYDM77
249	827447	Va1 [Homo sapiens] Length = 300		63	608			HAPNR75
250	827515	MLN 70, S100 C gene product [Homo sapiens] >gnl P1D1d1008178 calgizzarin [Homo sapiens]	gi 1698401	2	850	95	95	HTTED68
251	827621	>gnl P1D1d1008950 human S100C protein [Homo sapiens] >gi 2605598 calcium binding protein [Homo sapiens] >pir 37080 37080 calgizzarin - human Length = 105	gi 951233	42	443	100	100	HFNAC47
252	827883	(AF023269) probable transcriptional regulator drc4 [Drosophila melanogaster] >sp O17045 O17045 PROBABLE TRANSCRIPTIONAL REGULATOR DRE4 (FRAGMENT). Length = 1059		666	1004			HIIFHV82
253	828040		gi 2511745	54	1280	77	88	ITTEM37
254	828360	ADP-ribosylation factor 4 [Homo sapiens] >pir B38622 B38622 ADP-ribosylation factor 4 - human >sp P18085 ARF4_HUMAN ADP-RIBOSYLATION FACTOR 4. (SUB 2-180) >gnl P1D1e276443 ADP-ribosylation factor 4 [Sus scrofa] (SUB 1-51) Length = 180	gi 178985	2	292	100	100	HETFG60



255	828506	rac2 gene product [Canis familiaris] >gil190824 ras-related C3 botulinum toxin substrate [Homo sapiens] >gil53886 ras-related C3 botulinum toxin substrate [Mus musculus] >gil3184510 GTPase cRac1A [Gallus gallus] >pirA34788 TVHUC1 GTP-binding protein rac	gil922	182	319	100	100	HDQHC41
256	828517	p120E4F transcription factor [Homo sapiens] >sp O00146 O00146 P120E4F TRANSCRIPTION FACTOR. Length = 783	gil1906602	2	379	84	84	HPJDE77
257	828898	iron regulatory factor [Homo sapiens] >pir S26403 S26403 iron-responsive element-binding protein - human >sp P21399 IRE1_HUMAN IRON-RESPONSIVE ELEMENT BINDING PROTEIN 1 (IRE-BP 1) (IRON REGULATORY PROTEIN 1) (IRP1) (FERRITIN REPRESSOR PROTEIN) (ACONITATE	gil33963	2	1252	100	100	HMCBD26
258	828959	core protein II precursor [Homo sapiens]		1681	2115			HGBBQ46
259	829081	>pir A32629 A32629 ubiquinol--cytochrome-c reductase (EC 1.10.2.2) core protein II - human Length = 453	gil180928	3	953	88	88	IIQSF012
260	830069	(AC004492) HMG box containing protein 1 [Homo sapiens] >sp O60381 O60381 HMG BOX CONTAINING PROTEIN 1. Length = 514	gil2995607	3	1073	95	95	HDPBR82
261	830109			103	645			HCIAA79

262	830176	protein kinase C mu [Homo sapiens] >pir A53215 A53215 protein kinase C (EC 2.7.1.-) mu - human >sp Q15139 KPCM_HUMAN PROTEIN KINASE C, MU TYPE (EC 2.7.1.-) (NPKC-MU). Length = 912	gj 3438373	1	1131	86	92	HAPTB14
263	830241	(AF065482) sorting nexin 2 [Homo sapiens] >sp O60749 O60749 SORTING NEXIN 2. Length = 519	gj 3152938	1	1242	37	58	HTIEJV04
264	830264	transcription regulator helix-loop-helix protein=Id1 Id1-a [human, glioblastoma cell line U251, Peptide, 155 aa] [Homo sapiens] >gj 1816512 helix-loop- helix protein Id-1 [Homo sapiens] >pir JC5395 JC5395 helix-loop-helix protein Id1 - human Length = 155	bbs 169359	1	369	80	80	HAPTA45
265	830402	thromboxane synthase [Homo sapiens] >pir A41766 A41766 thromboxane-A synthase (EC 5.3.99.5) 1 - human Length = 534	gj 338704	1138 126	1482 1079	94	94	HWBEA34 HMCIR67
267	830444	mhc antigen DC-alpha chain [Homo sapiens] >gnl PID e307041 HLA-DQA1*05011 [Homo sapiens] >gj 2665521 MHC class II DC-alpha [Homo sapiens] >pir A02215 HLHU3C MHC class II histocompatibility antigen HLA-DQ alpha 1 chain precursor (allele DQA1*0501) - human	gj 307243	67	630	100	100	HTAAY31
268	830476	non-muscle myosin heavy chain [Bos taurus] >sp O02717 O02717 NON-MUSCLE MYOSIN HEAVY CHAIN (FRAGMENT). Length = 625	gj 3205211	1	1122	36	50	HWLHB64

269	830624	mRNA export protein [Homo sapiens] Length = 368	gil1903456	149	1387	100	100	HTEPA57
270	830643	CDC2-related kinase [Homo sapiens] >pirA55262/A55262 protein kinase (EC 2.7.1.37) cdc2-related PITALRE - human >spIP50750/CDK9_HUMAN CELL DIVISION PROTEIN KINASE 9 (EC 2.7.1.-) (SERINE/THREONINE-PROTEIN KINASE PITALRE) (C-2K). Length = 372	gil493130	1	1122	100	100	HTDAB49
271	830714	fibroblast growth factor receptor-FLG precursor [Homo sapiens] >gil31393 Fibroblast Growth Factor Receptor, 3-Ig Domain+2 AA insert [Homo sapiens] >gil35110 fibroblast growth factor receptor [Homo sapiens] >pirSI11692/TVHUFG fibroblast growth factor recep	gil31378	1	2292	95	95	HSSMW37
272	830826	plasminogen activator preprotein [Homo sapiens] >pirI38098/I38098 t-plasminogen activator (EC 3.4.21.68) precursor (variant) - human Length = 291	gil35283	114	593	92	92	HAJAC29
273	830888	von Ebner minor salivary gland protein [Mus musculus] >spIQ61114/Q61114 VON EBNER MINOR SALIVARY GLAND PROTEIN. Length = 310	gil1184790	1	663	64	81	HPSNE01
274	830984	antigen [Homo sapiens] >gil30949 pre-pro polypeptide (AA -22 to 163) [Homo sapiens] >pirS06786/A60592 T-cell surface glycoprotein E2 precursor - human >spP14209/MIC2_HUMAN T- CELL SURFACE GLYCOPROTEIN E2 PRECURSOR (E2 ANTIGEN) (CD99) (MIC2 PROTEIN) (12E7	gil188543	215	799	61	61	HOEKY01

275	831015	putative surface glycoprotein [Homo sapiens] >sp P53801 C211_HUMAN PUTATIVE SURFACE GLYCOPROTEIN C21ORF1 PRECURSOR (C21ORF3). Length = 180	gn P1D c 88111	79	633	88	88	HHEUT43
276	831080	lymphoma 3-encoded protein (bcl-3) [Homo sapiens] >pir A34794 A34794 B-cell CLL/lymphoma 3 (BCL3) protein - human >sp P20749 BCL3_HUMAN B-CELL LYMPHOMA 3-ENCODED PROTEIN (BCL-3 PROTEIN). >gi 533381 homologous to members of the I-kappa B family; protein bi	gi 179376	3	1703	77	83	HADDQ39
277	831101	zinc finger transcriptional regulator [Homo sapiens] >gi 340013 tristetraproline [Homo sapiens] >gi 183445 zinc finger transcriptional regulator [Homo sapiens] >pir S34427 S34427 tristetraproline protein - human Length = 326	gi 183443	3	899	66	66	HEOMO83
278	831146	(AF104670) cell cycle protein [Homo sapiens] >gi 4099506 erbB3 binding protein EBP1 [Homo sapiens] (SUB 55-394) Length = 394	gi 4160449	2	916	93	93	HDFTO32
279	831215	farnesyl-protein transferase alpha-subunit [Homo sapiens] >gi 388736 farnesyl-protein transferase alpha-subunit [Homo sapiens] >pir A47659 A47659 farnesyl-protein transferase alpha chain - human >sp P49354 PFTA_HUMAN PROTEIN FARNESYLTRANSFERASE ALPHA SUBU	gi 292031	1	1182	96	96	HMTAH30

280	831231	unknown orf, len: 393, CAl: 0.13 [Saccharomyces cerevisiae] >pir[S49759]S49759 probable membrane protein YML018c - yeast (Saccharomyces cerevisiae) Length = 393	gi 575701	2	1012	28	51	HMAIE05
281	831242	heat-shock protein HSP70B [Homo sapiens] >pir[S09036]S09036 dnaK-type molecular chaperone HSPA6 - human >sp P17066 HS76_HUMAN HEAT SHOCK 70 KD PROTEIN 6 (HEAT SHOCK 70 KD PROTEIN B'). >gi 35224 heat shock protein 70B' (AA 355-643) [Homo sapiens] {SUB 35	gi 35222	105	2099	91	91	HTTIV15
282	831267	glutathione peroxidase-G1 [Homo sapiens] Length = 190	gi 579930	246	464	99	99	HLTGF11
283	831272			641	1006			HLTDR01
284	831291			228	677			HLQER45
285	831382	epoxide hydrolase [Homo sapiens] Length = 455	gi 450269	541	1116	87	87	HFVHG01
286	831624	ornithine decarboxylase [Homo sapiens] >gi 386989 ornithine decarboxylase [Homo sapiens] >gi 338278 ornithine decarboxylase [Homo sapiens] >gi 338280 ornithine decarboxylase [Homo sapiens] >gi 35136 ornithine decarboxylase [Homo sapiens] >gi 296667 ornith	gi 189371	164	1561	100	100	HTC1HM53
287	831640	polypeptide BM28 [Homo sapiens] Length = 892	gi 468704	337	534	97	100	HFXHK31

288	831688	vascular anticoagulating protein [unidentified] >gil412271 VAC alpha [Mus musculus] >gil179132 anticoagulant precursor (S' end put.); putative [Homo sapiens] >gil307116 lipocortin-V [Homo sapiens] >gil182112 endonexin II [Homo sapiens] >gil37637 VAC prot	gil410788	164	787	100	100	HELPR81
289	831690	membrane cofactor preprotein (AA -34 to 350) [Homo sapiens] >pirS01896[S01896 membrane cofactor protein precursor - human >spG232300[G232300 MEMBRANE COFACTOR PROTEIN, MCP, CD46 {ALTERNATIVELY SPLICED}, {SUB 286-384} Length = 384	gil34505	46	1308	85	85	HOUHT44
290	831718	ets2 protein [Homo sapiens] >gil2736087 (AF017257) erythroleukemia virus oncogene homolog 2 protein [Homo sapiens] >pirB32066[TVHUE2 transcription factor ets-2 - human >spP15036[ETS2_HUMAN C-ETS-2 PROTEIN, >gil182271 ets protein [Homo sapiens] {SUB 324	gil182273	2	316	100	100	HFIXE61
291	831832	aldehyde dehydrogenase (NAD+) (EC 1.2.1.3) 2 precursor, mitochondrial - human >spP05091[DHAM_HUMAN ALDEHYDE DEHYDROGENASE, MITOCHONDRIAL PRECURSOR (EC 1.2.1.3) (CLASS 2) (ALDH1) (ALDH-E2), Length = 517	pirA29975[DEHUE2	881 92 54	1108 307 1289	95	95	HE8B04 HLHCN83 HDPXK32
292	831907							
293	831938							
294	831954							HDPTA79

295	832028	KM-102-derived reductase-like factor [Homo sapiens] >sp Q99475 Q99475 KM-102-DERIVED REDUCTASE-LIKE FACTOR. >gnl PID e1249328 (AJ001050) thioredoxin reductase [Homo sapiens] {SUB 53-549} Length = 549	gnl PID d1014370	494	1051	97	97	HDABD11
296	832043	similar to human 22kDa, SM22 mRNA (HUM22SM), [Homo sapiens] Length = 199	gnl PID d1005335	31	504	100	100	HTTAQ18
297	832055	calpastatin [Homo sapiens] Length = 464 Ubiquitin-conjugating enzyme UbcH2 [Homo sapiens] >gi 483538 ubiquitin-conjugating enzyme UbcH2 [Homo sapiens] >gi 897847 E2-20K [Mus musculus] >pir A53516 A53516 ubiquitin-conjugating enzyme UbcH2 - human >pir JC4308 JC4308 ubiquitin--protein ligase (EC	gi 951315 gi 474827	194	334			HCKQC81
298	832124			520	1155			HADCL25
299	832145			21	185			HCQDA34
300	832254			2	565			HCLBG05
301	832331			123	506			HBJBQ28
302	832360			89	811	83	83	HIFDI59
303	832401			1	558	90	90	HTEAB10
304	832403	adenylate kinase 2B [Homo sapiens] >pir JC5893 JC5893 adenylate kinase (EC 2.7.4.3) 2B - human Length = 232	gi 1477653	150	269			HAAPCK19
305	832437			1470	1664			HFIUK77
306	832492			2	430	100	100	HLJBH37

307	832598	CCAAT/enhancer binding protein gamma [Rattus norvegicus] >pir S26300 S26300 transcription factor C/EBP-gamma - rat (fragment) >sp P26801 CEBG_RAT CCAAT/ENHANCER BINDING PROTEIN GAMMA (C/EBP GAMMA). (SUB 86-235) Length = 235	gil55928	383	790	92	93	H2LAC64
308	832605	CRM1 protein [Homo sapiens] >sp O14980 O14980 CRM1 PROTEIN. Length = 1071	gil2626840	668	3322	99	99	H2CBU81
309	834510	macrophage inflammatory protein-1-alpha [Homo sapiens] >gil 179985 C-C chemokine receptor type 1 [Homo sapiens] >pir A45177 A45177 chemokine (C-C) receptor 1 - human >sp P32246 CCR1_HUMAN C-C CHEMOKINE RECEPTOR TYPE 1 (C-C CCR-1) (CC-CCR-1) (CCR-1) (CCR1)	gil292417	3	629	81	81	HMSGD45
310	835139	(AF106518) sialomucin CD164 [Homo sapiens] >sp G3941728 G3941728 SIALOMUCIN CD164. Length = 178	gil3941728	42	683	89	89	HAMF184
311	835142	(AF029213) IL-1 receptor accessory protein [Homo sapiens] >gnl PID d1026349 (A006537) interleukin 1 receptor accessory protein [Homo sapiens] >sp O14915 O14915 IL-1 RECEPTOR ACCESSORY PROTEIN. >gil2909775 (AF016261) interleukin-1 receptor accessory prote	gil2599127	3	1091	44	60	HJPDH20
312	835271	(AJ005766) LAMP [Homo sapiens] >sp E1363772 E1363772 LAMP PRECURSOR. Length = 416	gnl PID e1363772	3	698	90	90	HDQHB46



313	833369	melanoma-associated antigen [Homo sapiens] >gi34527 ME491 antigen precursor (AA -1 to 237) [Homo sapiens] >bbs93790 ocular melanoma-associated antigen, OMA81H [human, uveal melanoma, Peptide, 238 aa] [Homo sapiens] >gi430756 ME491 /CD63 antigen [Homo s	gi189384	3	386	87	87	HLJEA92
314	835430	(AF083236) FLDED-1 [Homo sapiens]		470	1240			HWBDL33
315	835462	>gnlPIDe1334489 (AJ010973) DEDD protein [Homo sapiens] >gi3930213 (AF043733) death effector domain-containing testicular molecule [Homo sapiens] >spO75618Q75618 FLDED-1. >spG3930213G3930213 DEATH EFFECTOR DOMAIN-CO	gi3462834	1	957	62	79	HLDOK36
316	835539	pre-mRNA splicing factor [Homo sapiens] >gi55440 X16 gene product [Mus musculus] >gnlPIDe274089 splicing factor [Mus musculus] >pirS14016[S14016 X16 protein - mouse >pir5408954089 pre-mRNA splicing factor - human Length = 164	gi338484	46	510	100	100	HWAAP51
317	835635	(AF097181) tuftelin-interacting protein 10 [Mus musculus] >gi3851164 (AF097181) tuftelin-interacting protein 10 [Mus musculus] >spG3851164G3851164 TUFTELIN-INTERACTING PROTEIN 10. Length = 526	gi3851164	2	2260	92	96	HDPKE84
318	835815	phosphate cyclase [Homo sapiens] >gnlPIDe311729 phosphate cyclase [Homo sapiens] (SUB 1-48) Length = 366	gnlPIDe311534	99	581	94	94	IISIAQ09

319	836161	mutant N-acetylglucosaminyltransferase I [Cricetulus griseus] Length = 447	gi 1331641	816	2288	48	68	HISCD15
320	836213	URF 3 (NADH dehydrogenase subunit) [Homo sapiens] >gi 506832 protein 3 [Homo sapiens] >pir A00422 DNHUN3 NADH dehydrogenase (ubiquinone) (EC 1.6.5.3) chain 3 - human mitochondrion (SGC1) >sp P03897 NU3M_HUMAN NADH-UBIQUINONE OXIDOREDUCTASE CHAIN 3 (EC 1.6	gi 13011	87	245	90	97	HAVMH34
321	836371	Similar to sulfatase [Caenorhabditis elegans] >sp Q21376 Q21376 SIMILAR TO SULFATASE. NCBI GI: 1125842. Length = 709	gi 1125842	3	530	60	74	HSYBS71
322	836618	(AF079446) developmental protein DG1067 [Dictyostelium discoideum] >sp O76738 O76738 DEVELOPMENTAL PROTEIN DG1067 (FRAGMENT). Length = 338	gi 3420747	76	1035	50	75	HWAGL94
323	836895	(AF039695) antigen NY-CO-25 [Homo sapiens] >sp G3170190 G3170190 ANTIGEN NY-CO-25 (FRAGMENT). >gnl PID id1013881 similar to mouse heat shock protein 105 kDa beta [Homo sapiens] (SUB 15-872) Length = 872	gi 3170190	2	2545	98	98	HNTCG15
324	837181			129	1046			HDTL355
325	837238	similar to DNAAJ [Caenorhabditis elegans] Length = 355	gnl PID e1349481	54	1265	58	78	HCHOH96
326	837337			1078	1446			HKABZ88

327	837530	prostaglandin transporter hPGT [Homo sapiens] >gi 3676522 (AF056732) prostaglandin transporter [Homo sapiens] >sp Q92959 PGT_HUMAN PROTAGLANDIN TRANSPORTER (PGT). >sp G367652 G3676522 PROTAGLANDIN TRANSPORTER. Length = 643	gi 1617590	2	517	43	72	HKIVF62
328	837551	(AF038960) SKD1 homolog [Homo sapiens] >sp O75351 O75351 SKD1 HOMOLOG. Length = 444	gi 3329390	2	1396	84	94	HEGAU95
329	837622	(AL008726) dJ337018.2 (Lysosomal Protective Protein precursor (EC 3.4.16.5, Cathepsin A, Carboxypeptidase C)) [Homo sapiens]	gnl PID c1296581	84	1484			HNFIB27
330	839908	>sp O60790 O60790 DJ337018.2 (LYSOSOMAL PROTECTIVE PROTEIN PRECURSOR (EC 3.4.16.5, CATHEPSIN A, CARBOXYPEPTIDASE C)). Length = 480		470	691			HTTFW42
331	839949			3	1478	100	100	HIDPRY42
332	840000	put. homologue to S.cerevisiae GAR1 gene [Drosophila melanogaster] >pir S49193 S49193 GCR 101 protein - fruit fly (Drosophila melanogaster)	gi 510509	489	1880			HPTXG77
333	840095	>sp Q24345 Q24345 GCR 101 MRNA. Length = 239		564	1580			HOEBZ29
334	840166			188	769	62	78	HILHEY06

335	840249	ATPase 6 [Homo sapiens] >gi2052364 ATPase 6 [Homo sapiens] >pirA01049 PWU6 H+-transporting ATP synthase (EC 3.6.1.34) protein 6 - human mitochondrion (SGC1) >sp P00846 ATP6_HUMAN ATP SYNTHASE A CHAIN (EC 3.6.1.34) (PROTEIN 6). Length = 226	gi 13009	520	870	65	68	HILJDL64
336	840601	integrin-linked kinase [Homo sapiens] >sp Q13418 Q13418 INTEGRIN-LINKED KINASE. Length = 452	gi 3150002	1	1287	99	99	HOEKB20
337	840613	(A1012463) transcription factor [Homo sapiens] >sp E1339598 E1339598 TRANSCRIPTION FACTOR. Length = 770	gn P1D1e1339598	126	2504	96	96	HARNB15
338	840699	t-complex polypeptide 1 (AA 1-556) [Homo sapiens] Length = 556	gi 36796	2	1015	94	95	HTENT25
339	840752							
340	840755	RAB14 [Rattus norvegicus] Length = 215	gi 206535	491	790	99	99	HDPDD66
341	840844			3	938			HIJFFM77
342	841066	J kappa RS-binding protein [Mus musculus] >bbs63468 J kappa recombination signal sequence binding protein, RBP-2 [mice, pre B cell line 38B9, Peptide, 526 aa] [Mus sp.] >pirA43567 A43567 J-kappa recombination sequence-binding protein - mouse >gi554133	gi 52757	1	219	96	96	HMELM17
				79	1629			H6EER20
343	841306	La/SS-B [Drosophila melanogaster] >pirA53773 A53773 La/SS-B homolog D-la - fruit fly (Drosophila melanogaster) >sp P40796 LA_DROME LA PROTEIN HOMOLOG (LA RIBONUCLEOPROTEIN) (LA AUTOANTIGEN HOMOLOG). Length = 390	gi 464020	64	696	51	76	HEMEU88
344	841913			44	217			HDPDC94

345	842025	prepromulimerin [Homo sapiens] >sp Q13201 ECM_HUMAN_ENDOTHELIAL CELL_MULTIMERIN_PRECURSOR. Length = 1228	gi 927596	2	1414	98	98	HE9OX09
346	842178	down syndrome candidate region 1; one of four alternatively spliced exon 1 [Homo sapiens]		3	986			HSKCF20
347	842438	>sp O00582 O00582_DOWN_SYNDROME CRITICAL_REGION_1_PROTEIN. >gi 2618743 down syndrome candidate region 1; one of four alternatively spliced exon 1 [Homo sapiens] {SUB	gi 2612866	3	428	98	100	HDTJH7
348	843289	(AF013249) leukocyte-associated Ig-like receptor-I [Homo sapiens] Length = 287	gi 2352941	334	1299	94	94	HMCFH47
349	843447	(AB000221) CC chemokine [Homo sapiens] >gnl PID c321838 CC-chemokine 1 [Homo sapiens] >gi 3426362 (AF082214) CC chemokine DC-CK- 1/PARC/MIP-4 [Homo sapiens] >gnl PID d1035349 (AB012113) CC chemokine PARC precursor [Homo sapiens] >sp P55774 MIP4_HUMAN_MACRO	gnl PID d1022520	42	332	100	100	HAPBV34
350	843743	gamma-interferon-inducible protein precursor [Homo sapiens] >pi A43708 A43708_gamma-interferon- inducible_protein_IP-30_precursor_human >sp P13284 INIP_HUMAN_GAMMA- INTERFERON-INDUCIBLE_PROTEIN_IP-30 PRECURSOR. Length = 303	gi 307042	3	803	88	88	HIMAKA82

351	843878	MHC class II DP3-alpha [Homo sapiens] >gil673417 class II antigen [Homo sapiens] >pirA29313 HLHUSB MHC class II histocompatibility antigen HLA-DP alpha-1 chain precursor - human >sp P20036 HA2Q_HUMAN HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DP ALPHA CHAIN	gil703089	42	932	76	76	HDPNI27
352	843964							
353	844071	TAR DNA-binding protein-43 [Homo sapiens] >pirI38977 I38977 TAR DNA-binding protein-43 - human >sp Q13148 Q13148 TAR DNA-BINDING PROTEIN-43. Length = 414	gil901998	318 125	656 1369	100	100	HSLDM31 HTDAI19
354	844444	Similar to man(9)-alpha-mannosidase [Caenorhabditis elegans] >sp Q22120 Q22120 SIMILAR TO MAN(9)-ALPHA-MANNOSIDASE. NCBI GI: 1086860. Length = 531	gil1086860	2	1828	53	73	HOUFX18
355	844561							
356	844953							
357	844990							
358	845379	similar to alcohol dehydrogenase/ribitol dehydrogenase [Caenorhabditis elegans] >sp Q09979 Q09979 HYPOTHETICAL 105.9 KD PROTEIN C17G10.8 IN CHROMOSOME II. Length = 938	gil2731377	3 1679 2 3	320 2020 1090 1745	52	74	HWAET73 HHSEF05 HCGMF32 IIDABUR2
359	845829	fatty aldehyde dehydrogenase [Homo sapiens] Length = 508	gil1666529	19	924	94	94	HOEJR65
360	HTAIR72R							
361	HAGDU65R	(AB020884) beta-actin [Plecoglossus altivelis] Length = 146	gnl PID d1036110	171 1	302 291	67	67	HTAIR72 HAGDU65

362	HAPRM14R	(AF000381) non-functional folate binding protein [Homo sapiens] >sp O14597 O14597 NON-FUNCTIONAL FOLATE BINDING PROTEIN. Length = 254	gi 2565196	183	377	88	88	HAPRM14
363	HMWEI22R	(AF012024) integrin cytoplasmic domain associated protein, lea-1b [Homo sapiens] >sp O14714 O14714 INTEGRIN CYTOPLASMIC DOMAIN ASSOCIATED PROTEIN. Length = 150	gi 2307002	176	349	100	100	HMWEI22
364	HE2IO29R	(AF018432) dUTPase [Homo sapiens] >gi 144332 deoxyuridine nucleotidohydrolase [Homo sapiens] >gi 1421818 deoxyuridine triphosphatase [Homo sapiens] >pir G02777 G02777 dUTP pyrophosphatase (EC 3.6.1.23) - human >gi 292877 dUTP nucleotidohydrolase [Homo sa	gi 2443581	2	190	100	100	HE2IO29
365	HE8QG48R	(AF059524) reticulon gene family protein [Homo sapiens] >sp G4091868 G4091868 RETICULON GENE FAMILY PROTEIN. Length = 236	gi 4091868	70	552	97	97	HE8QG48
366	HCLSJ64R	(AJ005368) SPR2J protein [Mus musculus] >sp O70561 O70561 SPR2J PROTEIN. Length = 109	gn p D e 1289139	3	185	59	67	HCLSJ64
367	HAMFN44R	acidic ribosomal phosphoprotein (P2) [Homo sapiens] >pir C27125 R6HUP2 acidic ribosomal protein P2 - human Length = 115	gi 190236	2	262	97	97	HAMFN44

368	HBNAZ1SR	adenine phosphoribosyltransferase [Homo sapiens] >gi28819 adenine phosphoribosyltransferase (aprt) [Homo sapiens] >pirS06232RTHUA adenine phosphoribosyltransferase (EC 2.4.2.7) - human >sp P07741 APT_HUMAN ADENINE PHOSPHORIBOSYLTRANSFERASE (EC 2.4.2.7)	gi178867	46	345	90	90	90	HBNAZ1S
369	HMC GG09R	apoferritin H chain [Homo sapiens] Length = 190	gi28435	3	257	100	100	100	HMC GG09
370	HADFF69R	ATP synthase, oligomycin sensitivity conferring protein [Homo sapiens] >sp P48047 ATPO_HUMAN ATP SYNTHASE OLIGOMYCIN SENSITIVITY CONFERRAL PROTEIN PRECURSOR, MITOCHONDRIAL (EC 3.6.1.34) (OSCP). >bbs165246 oligomycin sensitivity conferral protein oscp hom	gi1008080	2	265	88	88	88	HADFF69
371	HFPDJ19R	ATPase subunit 6 [Homo sapiens] >sp Q34772 Q34772 ATP SYNTHASE A CHAIN (EC 3.6.1.34). Length = 226	gnl P id1007873	139	537	63	63	66	HFPDJ19
372	H2MBA81R	autoantigen [Homo sapiens] >sp Q13823 NGP1_HUMAN AUTOANTIGEN NGP-1. Length = 731	gi179285	75	404	93	93	93	H2MBA81
373	HBGO121R	B-myb protein (AA 1-700) [Homo sapiens] >pirS01991 S01991 transforming protein B-myb - human >sp P10244 MYBB_HUMAN MYB- RELATED PROTEIN B (B-MYB). Length = 700	gi29472	3	368	100	100	100	HBGO121



374	HCLCW23R	carboxylesterase hCE-2 [Homo sapiens] >sp Q16859 Q16859 CARBOXYLESTERASE (EC 3.1.1.1) (ALI-ESTERASE) (B-ESTERASE) (MONOBUTYRASE) (COCAINE ESTERASE) (PROCAINE ESTERASE) (METHYLBUTYRASE). Length = 550	gil1407780	3	164	100	100	HCLCW23
375	HOSNF1IR	cytochrome oxidase I [Casuarinus bennettii] >sp O0352 COXI_CASBE CYTOCHROME C OXIDASE POLYPEPTIDE I (EC 1.9.3.1) (FRAGMENT). Length = 337	gil2198692	305	442	67	75	HOSNF1I
376	H2CAC1IR	elongation factor 1 alpha [Oryctolagus cuniculus] >gil1551 elongation factor 1 alpha [Oryctolagus cuniculus] >gil181963 elongation factor EF-1-alpha [Homo sapiens] >gil31098 EF-1 alpha (aa 1-463) [Homo sapiens] >pir B2497 EFHU1 translation elongation fac	gil495221	108	461	85	85	H2CAC1I
377	HBCCCK84R	exon [Homo sapiens] >pir 55360 55360 ornithine--oxo-acid transaminase (EC 2.6.1.13) - human (fragment) Length = 42	gil553605	174	413	93	93	HBCCCK84
378	HOEMQ09R	extracellular protein [Homo sapiens] >pir 38449 38449 extracellular protein - human >sp Q12805 Q12805 EXTRACELLULAR PROTEIN SI-5 PRECURSOR. Length = 387	gil458228	2	301	88	88	HOEMQ09
379	HMCHR51R	ferritin light subunit [Homo sapiens] >gil PID e284040 ferritin L-chain [Homo sapiens] (SUB 1-26) Length = 175	gil182518	2	490	74	75	HMCHR51

380	HALSF10R	fibrinogen gamma chain [Homo sapiens] >gij182439 fibrinogen gamma chain [Homo sapiens] >sp P02679 FIBG_HUMAN FIBRINOGEN GAMMA-A CHAIN PRECURSOR. >gij577055 gamma-fibrinogen chain fragment [Homo sapiens] {SUB 209-270} Length = 437	gij577054	3	188	85	86	HALSF10
381	HOEMK17R	fibronectin [Homo sapiens] >sp G4096860 G4096860 FIBRONECTIN (FRAGMENT). Length = 545	gij4096860	1	159	70	72	HOEMK17
382	HTLHA89R	GDP dissociation inhibitor [Homo sapiens] >gij456191 Human rho GDP-dissociation Inhibitor I (IEF 8118) [Homo sapiens] >gnl P1D1 003602 human rho GDI [Homo sapiens] >pir 38156 38156 rho protein GDP-dissociation inhibitor I (IEF 8118) - human >sp P52565 G	gij337395	3	80	100	100	HTLHA89
383	IIAGHZ15R	H-protein [Homo sapiens] >gnl P1D1 001083 hydrogen carrier protein precursor [Homo sapiens] >pir A36662 GCHUH glycine cleavage system protein H precursor - human >sp P23434 GCSH_HUMAN GLYCINE CLEAVAGE SYSTEM H PROTEIN PRECURSOR. Length = 173	gij184348	2	307	70	70	IIAGHZ15
384	HWAFE43R	hla-dr antigen alpha chain [Homo sapiens] >gij386945 HLA-DR alpha-chain [Homo sapiens] >gij307267 HLA-DR alpha-chain [Homo sapiens] >pir A93952 HLHUDA MHC class II histocompatibility antigen HLA-DR alpha chain precursor - human >sp P01903 HA2R_HUMAN HLA C	gij307264	3	422	93	93	HWAFE43

385	H2CAA26R	initiation factor 4B [Homo sapiens] >pir[S12566]S12566 translation initiation factor eIF-4B - human >sp[P23588]P4B_HUMAN EUKARYOTIC TRANSLATION INITIATION FACTOR 4B (EIF-4B). Length = 611	1	264	73	73	H2CAA26
386	HTLIW74R	inosine-5'-monophosphate dehydrogenase [Cricketulus griseus] >pir[B31997]B31997 IMP dehydrogenase (EC 1.1.1.205) - Chinese hamster >sp[P12269]MD2_MESAU INOSINE-5'- MONOPHOSPHATE DEHYDROGENASE 2 (EC 1.1.1.205) (IMP DEHYDROGENASE 2) (IMPDH- II) (IMPD 2). Le	2	652	95	95	HTLIW74
387	HDTHW36R		1	309			HDTHW36
388	HDPTT19R		36	278			HDPTT19
389	HKBAC11R		171	452			HKBAC11
390	HIBGOU32R	longest open reading frame [Homo sapiens] >sp[Q14617]Q14617 INTERFERON-INDUCIBLE MRNA (CDNA 1-8). Length = 152	1	231	80	80	HIBGOU32
391	HINTNC82R	Meis1-related protein 1b [Mus musculus] >gnl[P1D]e330084 (AJ000507) Homeodomain protein Meis2d [Mus musculus] Length = 470	1	438	98	98	HINTNC82
392	HMCIB16R	MHC class I histocompatibility antigen HLA-A3 alpha chain precursor - human >sp[P04439]IA03_HUMAN HLA CLASS I HISTOCOMPATIBILITY ANTIGEN, A-3 ALPHA CHAIN PRECURSOR. >gil187622 MHC class I antigen [Homo sapiens] (SUB 254-370) Length = 370	1	312	98	98	HMCIB16

393	HAPNX90R	MHC HLA-DQ-beta cell surface glycoprotein [Homo sapiens] >pir 55996 55996 MHC HLA-DQ-beta cell surface glycoprotein - human >sp Q30091 Q30091 MHC CLASS II HLA-DQ-BETA. Length = 261	gi 307255	227	376	100	100	HAPNX90
394	HSHAE55R	NF-kappa-B transcription factor subunit [Homo sapiens] >pir 53719 53719 NF-kappa-B transcription factor subunit - human >sp E78680 E78680 P65 SUBUNIT OF TRANSCRIPTION FACTOR NF-KAPPA-B. (SUB 281-331) Length = 337	gi 307300	3	188	90	95	HSHAE55
395	HJJBZ28R	nucleolar autoantigen No55 [Homo sapiens] >sp Q92791 Q92791 HUMAN NUCLEOLAR AUTOANTIGEN NO55. Length = 437	gi 1491809	2	316	98	100	HJJBZ28
396	HAGGW13R	placenta protein 9 [unidentified] >gi 179036 aldose reductase (EC 1.1.1.21) [Homo sapiens] >gi 178485 aldose reductase [Homo sapiens] >gi 178487 aldose reductase (EC 1.1.1.21) [Homo sapiens] >gi 178491 aldose reductase [Homo sapiens] >gi 28647 aldose reductase	gi 413094	1	147	75	78	HAGGW13
397	HAHDV81R	plasma gelsolin [Homo sapiens] >pir A03011 FAHUP gelsolin precursor, plasma - human >sp P06396 GELS_HUMAN GELSOLIN PRECURSOR, PLASMA (ACTIN-DEPOLYMERIZING FACTOR) (ADF) (BREVIN) (AGEL). >gn PID e20565 plasma gelsolin (AA 49-117) [Homo sapiens] (SUB 49-11)	gi 716249	3	302	92	95	HAHDV81

398	HACBP41R	plasma protease (C1) inhibitor precursor [Homo sapiens] Length = 500	gij179619	113	367	66	70	HACBP41
399	HANGC26R	plasma protease (C1) inhibitor precursor [Homo sapiens] Length = 500	gij179619	3	245	78	82	HANGC26
400	HESAN74R	platelet-derived growth factor receptor [Homo sapiens] >gij2107947 platelet-derived growth factor type beta receptor [Homo sapiens] [SUB 547-568] Length = 1106	gij189730	1	363	70	74	HESAN74
401	HWLMW20R			1	408			HWLMW20
402	HAPNU02R	pulmonary surfactant apoprotein precursor [Homo sapiens] Length = 248	gij190565	1	435	100	100	HAPNU02
403	HOUGB18R	put. eEF-TU (aa 1-94) [Homo sapiens] >gij50799 put. eEF-TU (aa 1-94) [Mus musculus] Length = 94	gij31110	20	202	71	73	HOUGB18
404	HBAGQ35R	put. lamin A precursor (aa 1-702) [Homo sapiens] Length = 702	gij34228	13	234	81	83	HBAGQ35
405	HADME37R	pyruvate kinase (EC 2.7.1.40), muscle splice form M1 - human Length = 531	pir[S64635]S64635	16	135	100	100	HADME37
406	HAPQM68R	raf protein (aa 1-648) [Homo sapiens] >pir[A00637]TVHUF6 protein kinase raf-1 (EC 2.7.1.-) - human >sp P04049 KRAF_HUMAN RAF PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE (EC 2.7.1.-) (RAF-1) (C-RAF). >gij2257953 (AF006463) c-RAF homolog [Papio hamadryus]	gij35842	53	427	99	99	HAPQM68
407	HSLGI35R	ribosomal protein S28 [Homo sapiens] >gij337403 ribosomal protein S28 [Homo sapiens] >gij508266 ribosomal protein S28 [Mus musculus] >gij57726 ribosomal protein S28 [Rattus rattus] >gij4050094 (AF110520) RPS28 [Mus musculus] >pir Q1170 R3RT28 ribosomal p	gij1518637	3	158	93	93	HSLGI35

408	HDPQN35R	signal recognition particle, 72 kDa subunit [Canis familiaris] >pir A40692 A40692 signal recognition particle 72K chain - dog Length = 671	gi 297768	2	325	93	95	HDPQN35
409	HAPNU41R	SP-A2 delta=surfactant protein (N-terminal, alternatively spliced) [human, fetal lung explants, Peptide Partial, 41 aa] [Homo sapiens] >pir 64840 64840 SP-A2 delta - human (fragment) >sp Q16139 Q16139 SP-A2 DELTA (FRAGMENT), Length = 41	bbs 146432	3	308	100	100	HAPNU41
410	HSYCT58R	tenascin [Homo sapiens] Length = 2199	gi 37227	2	148	100	100	HSYCT58
411	HFKLT54R	tissue-specific secretory protein [unidentified] >gi 818881 epididymal secretory protein precursor [Pan troglodytes] >gi 794071 epididymal secretory protein 14.6 [Macaca fascicularis] >gi 37477 orf [Homo sapiens] >pir 53929 53929 epididymal secretory pr	gi 513467	3	392	84	84	HFKLT54
412	HTXNT90R	transfer RNA-Tyr synthetase [Homo sapiens] >gi 30821 471 aa polypeptide (gamma2) [Homo sapiens] >pir A41633 A41706 tryptophan-tRNA ligase (EC 6.1.1.2) - human >bbs 179357 tryptophanyl-tRNA synthetase, TrpRS (N-terminal, alternatively spliced) {EC 6.1.1.2	gi 184657	3	521	100	100	HTXNT90
413	H6BSD14R			123	539			H6BSD14
414	H6EEC47R			1	90			H6EEC47
415	HACBQ15R			279	452			HACBQ15
416	HADTH59R			2	208			HADTH59
417	HAIAA21R			1	144			HAIAA21
418	HANGG63R			3	182			HANGG63

419	HAPAK90R	66	227	HAPAK90
420	HAPBH25R	318	506	HAPBH25
421	HAPBP34R	3	236	HAPBP34
422	HAPBV57R	180	380	HAPBV57
423	HAIPOL49R	80	316	HAIPOL49
424	HAPQO76R	3	350	HAPQO76
425	HBAFB37R	247	417	HBAFB37
426	HBKDI63R	140	259	HBKDI63
427	HCFLU89R	298	411	HCFLU89
428	HCLCX30R	92	376	HCLCX30
429	HCUCD74R	2	91	HCUCD74
430	HDTFW96R	63	290	HDTFW96
431	HDTLW91R	406	588	HDTLW91
432	HE9GW86R	59	289	HE9GW86
433	HFACI43R	1	123	HFACI43
434	HHBGW74R	196	330	HHBGW74
435	HHFLJ48R	3	323	HHFLJ48
436	HHFLJ50R	240	425	HHFLJ50
437	HKMMF86R	1	75	HKMMF86
438	HMCIB02R	212	424	HMCIB02
439	HOEKC43R	1	381	HOEKC43
440	HPJCZ62R	34	231	HPJCZ62
441	HPJDY23R	37	84	HPJDY23
442	HSXEN17R	244	354	HSXEN17
443	HMC GG17R	1	273	HMC GG17

vacuolar H<sup>+</sup>-ATPase B subunit [Gallus gallus]  
Length = 453

gi|675489

100

100

The first column of Table 1 shows the "SEQ ID NO:" for each of the 443 lung cancer antigen polynucleotide sequences of the invention.

The second column in Table 1, provides a unique "Sequence/Contig ID" identification for each lung and/or lung cancer associated sequence. The third column in Table 1, "Gene Name," provides a putative identification of the gene based on the sequence similarity of its translation product to an amino acid sequence found in a publicly accessible gene database, such as GenBank (NCBI). The great majority of the cDNA sequences reported in Table 1 are unrelated to any sequences previously described in the literature. The fourth column, in Table 1, "Overlap," provides the database accession no. for the database sequence having similarity. The fifth and sixth columns in Table 1 provide the location (nucleotide position nos. within the contig), "Start" and "End", in the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred ORF shown in the sequence listing as SEQ ID NO:Y. In one embodiment, the invention provides a protein comprising, or alternatively consisting of, a polypeptide encoded by the portion of SEQ ID NO:X delineated by the nucleotide position nos. "Start" and "End". Also provided are polynucleotides encoding such proteins and the complementary strand thereto. The seventh and eighth columns provide the "% Identity" (percent identity) and "% Similarity" (percent similarity) observed between the aligned sequence segments of the translation product of SEQ ID NO:X and the database sequence.

The ninth column of Table 1 provides a unique "Clone ID" for a clone related to each contig sequence. This clone ID references the cDNA clone which contains at least the 5' most sequence of the assembled contig and at least a portion of SEQ ID NO:X was determined by directly sequencing the referenced clone. The reference clone may have more sequence than described in the sequence listing or the clone may have less. In the vast majority of cases, however, the clone is believed to encode a full-length polypeptide. In the case where a clone is not full-length, a full-length cDNA can be obtained by methods described elsewhere herein.

Table 3 indicates public ESTs, of which at least one, two, three, four, five, ten, or more of any one or more of these public ESTs are optionally excluded from the invention.

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing as SEQ ID NO:1 through SEQ ID NO:443) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing as SEQ ID NO:444 through SEQ ID NO:886) are sufficiently accurate and otherwise suitable for a



variety of uses well known in the art and described further below. For instance, SEQ ID NO:X has uses including, but not limited to, in designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the related cDNA clone contained in a library deposited with the ATCC. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling immediate applications in chromosome mapping, linkage analysis, tissue identification and/or typing, and a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y have uses that include, but are not limited to, generating antibodies which bind specifically to the lung cancer antigen polypeptides, or fragments thereof, and/or to the lung cancer antigen polypeptides encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X, the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing the related cDNA clone (deposited with the ATCC, as set forth in Table 1). The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. Further, techniques known in the art can be used to verify the nucleotide sequences of SEQ ID NO:X.

The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to vectors or plasmids which include such DNA sequences, as well as the use of the DNA sequences. The material deposited with the ATCC on:

5 Table 2

ATCC Deposits	Deposit Date	ATCC Designation Number
LP01, LP02, LP03, LP04, LP05, LP06, LP07, LP08, LP09, LP10, LP11,	May-20-97	209059, 209060, 209061, 209062, 209063, 209064, 209065, 209066, 209067, 209068, 209069
LP12	Jan-12-98	209579
LP13	Jan-12-98	209578
LP14	Jul-16-98	203067
LP15	Jul-16-98	203068
LP16	Feb-1-99	203609
LP17	Feb-1-99	203610
LP20	Nov-17-98	203485
LP21	Jun-18-99	PTA-252
LP22	Jun-18-99	PTA-253
LP23	Dec-22-99	PTA-1081

each is a mixture of cDNA clones derived from a variety of human tissue and cloned in either a plasmid vector or a phage vector, as shown in Table 5. These deposits are referred to as "the deposits" herein. The tissues from which the clones were derived are listed in Table 5, and the vector in which the cDNA is contained is also indicated in Table 5. The deposited material includes the cDNA clones which were partially sequenced and are related to the SEQ ID NO:X described in Table 1 (column 9). Thus, a clone which is isolatable from the ATCC Deposits by use of a sequence listed as SEQ ID NO:X may include the entire coding region of a human gene or in other cases such clone may include a substantial portion of the coding region of a human gene. Although the sequence listing lists only a portion of the DNA sequence in a clone included in the ATCC Deposits, it is well within the ability of one skilled in the art to complete the sequence of the DNA included in a clone isolatable from the

ATCC Deposits by use of a sequence (or portion thereof) listed in Table 1 by procedures hereinafter further described, and others apparent to those skilled in the art.

Also provided in Table 5 is the name of the vector which contains the cDNA clone. Each vector is routinely used in the art. The following additional information is provided for convenience.

5 Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res.* 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., *Nucleic Acids Res.* 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., *Strategies* 5:58-61 (1992)) are commercially available from Stratagene  
10 Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Phagemid pBS may be excised from the Lambda Zap and Uni-Zap XR vectors, and phagemid pBK may be excised from the Zap Express vector. Both phagemids may be transformed into *E. coli* strain  
15 XL-1 Blue, also available from Stratagene.

Vectors pSport1, pCMVSPORT 1.0, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus*  
20 15:59 (1993). Vector lafmid BA (Bento Soares, Columbia University, New York, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res.*  
25 16:9677-9686 (1988) and Mead, D. et al., *Bio/Technology* 9: (1991).

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the cDNA contained in a deposited cDNA clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include, but are not limited to, preparing probes or primers from the  
30 disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the cDNA contained in the related cDNA clone in the deposit, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or the related cDNA clone (See, e.g., columns 1 and 9 of Table 1). The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in a deposited library. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the the dDNA in the related cDNA clone contained in a deposited library, are also encompassed by the invention. The present invention further encompasses a polynucleotide comprising, or alternatively consisting of, the complement of the nucleic acid sequence of SEQ ID NO:X, and/or the complement of the coding strand of the related cDNA clone contained in a deposited library.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would unduly burden the disclosure of this application. Accordingly, for each "Contig Id" listed in the first column of Table 3, preferably excluded are one or more polynucleotides comprising a nucleotide sequence described in the second column of Table 3 by the general formula of a-b, each of which are uniquely defined for the SEQ ID NO:X corresponding to that Contig Id in Table 1. Additionally, specific embodiments are directed to polynucleotide sequences excluding at least one, two, three, four, five, ten, or more of the specific polynucleotide sequences referenced by the Genbank Accession No. for each Contig Id which may be

included in column 3 of Table 3. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example.

Table 3.

Sequence/ Contig ID	General formula	Genbank Accession No.
507002	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1566 of SEQ ID NO:1, b is an integer of 15 to 1580, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1, and where b is greater than or equal to a + 14.	
508935	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2428 of SEQ ID NO:2, b is an integer of 15 to 2442, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:2, and where b is greater than or equal to a + 14.	
518959	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1773 of SEQ ID NO:3, b is an integer of 15 to 1787, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:3, and where b is greater than or equal to a + 14.	R12691, R16433, W06913, AA253226, AA458465
539756	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 832 of SEQ ID NO:4, b is an integer of 15 to 846, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:4, and where b is greater than or equal to a + 14.	T85355, T85452, R01748, R01749, R21683, R76830, R76831, R81140, R81139, N41431, N99543, W92271
540125	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1263 of SEQ ID NO:5, b is an integer of 15 to 1277, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:5, and where b is greater than or equal to a + 14.	N30606, N40661, N41850, N42208, N64348, AA261980
540275	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2188 of SEQ ID NO:6, b is an integer of 15 to 2202, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:6, and where b is greater than or equal to a + 14.	T58888, T58950, T77750, T77751, R11911, R02521, R14197, R15120, R17948, R22687, R37480, R39103, R41544, R43393, R43438, R41544, R43393, R43438, H45141, R83378, R83819, R86096, H49887, H65432, H65433, H94973, H95493, H65433, H98864, H99146, N24395, N27550,

	14.	N40365, N46111, N47507, N47508, N55276, N62977, N76885, W45533, W45520, W67533, W67534, W80460, N89761, AA011245, AA100471, AA101453, AA135125, AA135238, AA204704, AA227873, AA227874
540331	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1284 of SEQ ID NO:7, b is an integer of 15 to 1298, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:7, and where b is greater than or equal to a + 14.	T60064, T62000, T39218, T39235, T39248, T63485, T63560, T63653, T63834, T63850, T63908, T63969, T92745, T92902, T94295, T94457, T94546
540955	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1749 of SEQ ID NO:8, b is an integer of 15 to 1763, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:8, and where b is greater than or equal to a + 14.	T47346, T39490, T49501, T53352, T53353, T58751, T59830, T62508, T70053, T70119, T71663, T71816, T92710, T92786, T70242, T87877, T87967, T89395, T89753, R00830, R01486, R22448, R22500, R24884, R53792, R68690, R68745, R70643, R81546, R81545, R81829, R82028, R82074, H01094, H03335, H12560, H13083, H13287, H70273, H95157, H95199, N22040, N26996, N40117, N53786, N54556, N69444, N73148, N76636, N93982, W52688, W74093, W79383, W94662, W96029, AA001255, AA001108, AA002260, AA001637, AA010621, AA010622, AA031960, AA032042, AA057472, AA069313, AA074511, AA100094, AA224261
541251	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2141 of SEQ ID NO:9, b is an integer of 15 to 2155, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:9, and where b is greater than or equal to a + 14.	T89145, T89238, T66188, T79629, T79714, R06893, R06935, R17707, R33911, R59938, R59939, H70419, H88949, H89182, H88949, H99641, N24430, N93479, W21497, AA047879, AA069880, AA070006, AA113048, AA113388, AA112639, AA127456, AA169143, AA169235, AA182036, AA188210, AA186357, AA188395, AA192379, AA197275, AA223748, AA464825
541978	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1194 of SEQ ID NO:10, b is an integer of 15 to 1208, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:10, and where b is greater than or equal to a + 14.	
547680	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	T49230, T48210, T49231, R87539, W02155, W02798, W07585, W30953, W32489, W47384, W47256, W68528,

	formula of a-b, where a is any integer between 1 to 2298 of SEQ ID NO:11, b is an integer of 15 to 2312, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.	W68814, W70219, W70220, W78133, W79471, W81199, W86008, W95376, AA027019, AA040292, AA040533, AA040756, AA041210, AA043912, AA157956, AA186594, AA459745, AA461327
547705	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 901 of SEQ ID NO:12, b is an integer of 15 to 915, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.	T70761, R11269, H57226, N28016, N41991, W31920, AA224454
549763	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1438 of SEQ ID NO:13, b is an integer of 15 to 1452, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.	T77113, T86571, T86749, H47480, R89205, H56994, H61812, N20326, N27600, N31373, N36157, N74455, N93384, W03169, W25403, W77860, AA029438, AA029503, AA052974, AA053468, AA053190, AA054520, AA057649, AA057748, AA071554, AA113040, AA112548, AA112612, AA179799, AA227845, AA227846
549819	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 427 of SEQ ID NO:14, b is an integer of 15 to 441, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.	
549820	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 510 of SEQ ID NO:15, b is an integer of 15 to 524, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.	
549944	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2418 of SEQ ID NO:16, b is an integer of 15 to 2432, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.	W05270, AA193211
551426	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 358 of SEQ ID NO:17, b is an integer of 15 to 372, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.	
552182	Preferably excluded from the present invention are	



	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 915 of SEQ ID NO:18, b is an integer of 15 to 929, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.	
552540	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 402 of SEQ ID NO:19, b is an integer of 15 to 416, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.	
553367	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1839 of SEQ ID NO:20, b is an integer of 15 to 1853, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.	R46353, R46444, R49217, R49217, R69441, R70422, H27076, R85073, N69960, N93506, W21318, W92281
554326	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1693 of SEQ ID NO:21, b is an integer of 15 to 1707, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a + 14.	T64826, R10203, T97648, T97682, H24794, H38748, H84580, N42355
554657	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 856 of SEQ ID NO:22, b is an integer of 15 to 870, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.	T54995, T55161, T57268, R08423, R99052, R99250, H60049, H63552, H63597, H67322, H68531, N36577, N51896, N55541, N62719, N73182, N78443, N78711, W32492, W37491, W37385, W81233, W81269, AA025552, AA025653, AA099004, AA099073, AA128876, AA155680, AA155970, AA159986, AA159987, AA176848, AA232955, AA232999, AA233045, AA464479
556156	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 640 of SEQ ID NO:23, b is an integer of 15 to 654, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.	R11164, R12444, R15118, R24789, H00898, N29154, N42711, W00889, W17263, W44321, AA005179, AA100065, AA111892, AA158702, AA158780, AA158829
557747	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1386 of SEQ ID NO:24, b is an integer of 15 to	T61816, T67403, T73342, T73411, T89475, T89568, H47837, H47838, H61865, N54494, N63924, W89198

	1400, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.	
558599	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 629 of SEQ ID NO:25, b is an integer of 15 to 643, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.	T71049, T71118, T95493, T99242, T99287, H93555, H93556, N39552, N45356, N53827, N58306, N58495, N68310, N69477, N73323, N74608, N77672, N78084, W00883, N90969, AA009570, AA081234, AA128611, AA128612, AA130716, AA130801, AA132483, AA132572, AA132932, AA147273, AA147330, AA169473, AA196031, AA196060
572403	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1117 of SEQ ID NO:26, b is an integer of 15 to 1131, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.	
573366	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 150 of SEQ ID NO:27, b is an integer of 15 to 164, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.	
573986	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 646 of SEQ ID NO:28, b is an integer of 15 to 660, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.	
575435	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3122 of SEQ ID NO:29, b is an integer of 15 to 3136, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.	T39164, T39174, T40441, T40449, T40455, T51400, T69999, T93268, T94124, T94213, T94325, T94678, T96580, T96579, T98149, T90974, R08466, T97330, R01057, R21527, R21631, R28044, R32284, R33571, R33673, R40061, R44440, R44440, R55126, R55173, R63491, R63492, R64700, R68535, R72421, R72465, R74196, R77928, R78031, R80572, R82486, R82538, H15529, H15586, H20497, H20783, H21578, H25948, H25983, H42024, H42475, H42731, H43022, H43458, H47778, H48123, R88742, R89796, R90999, R93140, R93577, R93578, H48221, H48313, H53603, H54320, H54458, H57514, H57515, H58276, H58669, H62236.

		H64556, H64657, H68468, H68832, H73763, H74146, H79745, H79746, H79951, H79952, H88625, H89320, H89321, H64556, H99470, N20526, N20609, N21014, N22983, N26530, N28451, N29225, N30281, N31609, N32687, N34439, N35641, N42003, N42669, N46265, N46266, N63160, N68956, N76751, N79616, N91859, N91867, N91875, N93308, W00656, W02210, W05662, W19094, W19690, W19766, W20234, W23909, W23920, W23766, W24656, W25160, W32603, W32899, W35295, W37538, W37539, W61273, W61274, W61286, W65351, W69441, W69454, W69524, W69538, W69984, W72634, W88520, W90420, W93058, W93181, W93652, W93769, W92524, W95223, N90036, N90596, N91354, AA004315, AA005187, AA010768, AA011049, AA025845, AA028943, AA028944, AA036885, AA043676, AA044019, AA044194, AA044633, AA044760, AA045805, AA045872, AA046059, AA046185, AA053688, AA055602, AA058395, AA069616, AA070803, AA070877, AA076618, AA076619, AA081635, AA086013, AA086001, AA086108, AA088405, AA088528, AA098990, AA099052, AA098968, AA099486, AA112997, AA134382, AA134394, AA132038, AA131966, AA135178, AA135219, AA135352, AA135368, AA136149, AA136227, AA142955, AA147754, AA148934, AA148935, AA150462, AA161240, AA159691, AA159622, AA167108, AA167305, AA169502, AA176422, AA178843, AA180747, AA182407, AA182750, AA192405, AA192259, AA193226, AA194713, AA194777, AA253456, AA250911, AA463349, AA463448, AA463857
584341	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2234 of SEQ ID NO:30, b is an integer of 15 to 2248, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.	T57793, T60105, T60148, T60207, T39185, T62531, T62548, T62606, T63248, T63308, T63963, T64488, T64631, T65789, T65906, T65911, T70024, T72012, T82355, T91763, T92526, T93717, T94845, T94890, T96181, H64702, H65194, H65194
584435	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	R37836, R37922, R97973, R97974, H57703, H65855, H65859, H65860, H70124, H70125, H84404, N26402, N74594, W02493, AA054124,

	2033 of SEQ ID NO:31, b is an integer of 15 to 2047, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.	AA054169, AA054189, AA115229, AA132090, AA132191
585187	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1821 of SEQ ID NO:32, b is an integer of 15 to 1835, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.	
585658	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1285 of SEQ ID NO:33, b is an integer of 15 to 1299, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.	T66734, R14942, AA171576, AA196708
585693	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3326 of SEQ ID NO:34, b is an integer of 15 to 3340, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.	T63022, T72992, T73117, R08174, T95097, T95197, T96940, T97049, R22071, R22459, R26391, R33251, R36336, R53795, R68262, R68293, R76264, R78125, R78126, R82269, R82381, H01353, R95875, R95876, H57149, H57223, N30812, N41586, N53545, N76004, W04423, W93076, W93075, AA002005, AA001726, AA035777, AA056355, AA082477, AA151818, AA151817, AA181092, AA181110, AA181062, AA181063, AA458728
585701	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1476 of SEQ ID NO:35, b is an integer of 15 to 1490, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.	
586019	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2841 of SEQ ID NO:36, b is an integer of 15 to 2855, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.	
587225	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	R53038

	formula of a-b, where a is any integer between 1 to 976 of SEQ ID NO:37, b is an integer of 15 to 990, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.	
587445	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 419 of SEQ ID NO:38, b is an integer of 15 to 433, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.	T63800, T92291, T93032
587572	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 912 of SEQ ID NO:39, b is an integer of 15 to 926, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.	T39188, T57086, T57157, T57684, T59683, T59819, T61442, T40462, T63153, T63392, T63463, T63492, T63567, T63614, T64088, T64267, T64475, T64616, T65748, T65823, T65888, T65969, T69898, T70225, T91680, T91712, T91718, T92174, T92207, T92485, T94608, T94930, T96156, T96416, H64383, H67218, H81539, H81539, N75529, N78904, N79800, N81037, N92395, W05322, W07040, W17198, W19062, W21038, W38307, W57819, W58196, W58305, W73016, W74518, W76624, W79858, W94532, W94533, N91554
587596	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 392 of SEQ ID NO:40, b is an integer of 15 to 406, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.	
588548	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1487 of SEQ ID NO:41, b is an integer of 15 to 1501, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.	R08817, R05288, R72561
588881	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1560 of SEQ ID NO:42, b is an integer of 15 to 1574, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.	T53536, T53537, T68114, T68227, T72810, T74028, T79915, R15360, T27002, T27019, T27020, R42103, R42103, R69022, R69023, R77749, R78100, R80740, R80741, H11719, H11791, H12816, H21355, H21454, H39598, H52177, H52589, N58960, N63521, N99113, W56632, W56546, W79086, W86574, W86708, W87269, W87270, AA004445, AA004448, AA041274, AA041190, AA128152, AA125916, AA151162, AA151161.

588933	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2182 of SEQ ID NO:43, b is an integer of 15 to 2196, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a + 14.	AA158853, AA161117, AA161123 T53947, T57930, T58014, T61642, T62086, T62144, T67707, T67783, T68996, T72216, T91400, T84630, H40325, R83041, R83100, R86262, R86263, R91939, R92267, R94960, R95045, R96200, R96250, R98807, R99812, R99811, H48384, H57704, H57705, H58864, H59880, H59881, H61691, H61692, H62677, H62706, H62791, H62807, H65307, H65322, H65520, H70399, H71391, H72055, H72124, H72143, H72227, H90149, H90251, H93798, H93853, H94656, H94657, N52576, N68290, N69996, N72344, N80987, W00805, W04945
592136	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3771 of SEQ ID NO:44, b is an integer of 15 to 3785, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.	T56435, T56586, R12185, H73242, H74170, H97928, H99752, N22702, N28469, N28723, N29119, N40389, N44822, N64243, N72873, N72909, W03094, W02900, AA029776, AA029777, AA043141, AA075877, AA150621, AA234264, AA262981
613777	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 466 of SEQ ID NO:45, b is an integer of 15 to 480, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a + 14.	
614669	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 996 of SEQ ID NO:46, b is an integer of 15 to 1010, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:46, and where b is greater than or equal to a + 14.	T40168, T79688, R13509, R17640, R21689, H18568, W25423, W39596, W53031, AA082508, AA146679
619502	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3759 of SEQ ID NO:47, b is an integer of 15 to 3773, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a + 14.	T57882, T57964, R35719, R35828, R61628, H12952, H42833, H45734, H84427, N41844, W39544, AA039905, AA233571, AA233683
619525	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1448 of SEQ ID NO:48, b is an integer of 15 to 1462, where both a and b correspond to the	T39535, T74055, R39623, R62868, R64651, R65629, H03029, H40547, R97358, H60900, H67966, H71201, H72546, H77491, H79590, H81459, N72440, N77701, AA221001, AA227317

	positions of nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.	
623660	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 547 of SEQ ID NO:49, b is an integer of 15 to 561, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where b is greater than or equal to a + 14.	R25689, H80051, W38939, W78791, AA055158, AA099749, AA113422, AA113804, AA115779, AA182431, AA182755, AA188531, AA190692, AA224428
625480	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1197 of SEQ ID NO:50, b is an integer of 15 to 1211, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where b is greater than or equal to a + 14.	R51936, H77807
647688	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1586 of SEQ ID NO:51, b is an integer of 15 to 1600, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a + 14.	AA054968, AA071510, AA236671
650865	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1554 of SEQ ID NO:52, b is an integer of 15 to 1568, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.	T84055, W69878, AA112954, AA255796, AA258551, AA463341, AA424719
651676	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1029 of SEQ ID NO:53, b is an integer of 15 to 1043, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.	R50184, R50238, R75668, H13196, H13197, R86027, R93619, R93620, H53347, H53384, H53639, H53682, N95154, W21530, W32438, W45012, AA025096, AA025186, AA036986, AA036987, AA044584, AA054519, AA054772, AA074644, AA079479, AA079480, AA223158, AA223436, AA464687, AA424138, AA429026, AA429198, AA429239, AA427581, AA427630
651751	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2557 of SEQ ID NO:54, b is an integer of 15 to 2571, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a +	T39956, T50706, T50861, T71880, N79771, N93428, W07270, W19106, W21470, AA233679

	14.	
651787	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1288 of SEQ ID NO:55, b is an integer of 15 to 1302, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a + 14.	T59677, T59813, T63514, T64074, T69968, T73846, T74246, T89719, T89816, T89830, T92638, T92944, T93011, T93218, T98228, T85518, R47979, R48091, R56246, R56328, R73988, H05307, H05357, H24508, H47709, H47714, H48061, H48066, R96654, R96706, H51095, H51680, H51999, H52596, H52597, H78841, H80088, H82407, H82665, N41665, N54547, N69295, N76250, N80222, N92452, N94117, N98344, N98394, W31336, W38511, W40187, W40190, N90939, AA024976, AA025072, AA046296, AA046360, AA075540, AA075553, AA075663, AA075664, AA075729, AA075781, AA158653, AA159063, AA164191, AA164192, AA166826, AA166997, AA167336, AA169607, AA236638, AA463953, AA464089, AA429853, AA429884
651840	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1423 of SEQ ID NO:56, b is an integer of 15 to 1437, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:56, and where b is greater than or equal to a + 14.	T66896, W07604, AA011006, AA025714, AA099639, AA102243, AA100320, AA099769, AA100565, AA112731, AA112732, AA112721, AA112780, AA112793, AA112800, AA112810, AA112826, AA112832, AA111913, AA112038, AA112790, AA112836, AA112948, AA112949, AA113000, AA128476, AA176521, AA176789, AA176673, AA178866, AA178904, AA179111, AA179247, AA179122, AA179972, AA180244, AA180980, AA180803, AA182902, AA178919, AA192575, AA192585, AA192264, AA192710, AA193009, AA193072, AA193208, AA194449, AA194460, AA194479, AA194342, AA195969, AA196021, AA196089, AA196097, AA196120, AA196364, AA196366, AA196653, AA196642, AA196673, AA196681, AA196846, AA197130, AA197163, AA197239
651892	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2019 of SEQ ID NO:57, b is an integer of 15 to 2033, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:57, and where b is greater than or equal to a + 14.	
652557	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	



	1818 of SEQ ID NO:58, b is an integer of 15 to 1832, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where b is greater than or equal to a + 14.	
653011	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1392 of SEQ ID NO:59, b is an integer of 15 to 1406, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where b is greater than or equal to a + 14.	T60269, T61649, T64306, R34841, R38709, R43326, R47775, R43326, R76527, R80595, R80596, H04312, H04354, H27791, R98843, H69074, N31337, N33901, N49898, N62500, N67530, W78148, W79913, AA128245, AA234063, AA234454, AA253399, AA253502
656155	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 251 of SEQ ID NO:60, b is an integer of 15 to 265, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:60, and where b is greater than or equal to a + 14.	
656930	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 923 of SEQ ID NO:61, b is an integer of 15 to 937, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:61, and where b is greater than or equal to a + 14.	
659023	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 698 of SEQ ID NO:62, b is an integer of 15 to 712, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:62, and where b is greater than or equal to a + 14.	AA024848
659263	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1044 of SEQ ID NO:63, b is an integer of 15 to 1058, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:63, and where b is greater than or equal to a + 14.	
660696	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2677 of SEQ ID NO:64, b is an integer of 15 to 2691, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:64, and where b is greater than or equal to a + 14.	T47468, T47696, T48602, T48603, T49199, T49200, T49659, T49660, T49673, T49674, T51475, T56701, T60767, T60810, T91749, T92514, T70778, T90173, R09881, T77979, T78190, T78323, T78702, T80415, T81934, T81935, T82983, T97143, R22979, R23085, R23454, R23465, R23985, R24181, R24474, R24475, R26053, R26062, R26967, R28422.

		R31303, R31565, R33106, R33203, R35991, R36364, R36676, R37192, R37193, R39689, R51191, R53583, R62740, R62965, R63019, R63693, R63717, R63763, R64121, R64219, R66523, R68974, R68975, R70114, R70115, R72983, R73586, R73594, R78225, R78226, H01045, H01425, H01426, H01798, H40591, H45106, H45415, R86177, R86338, R92741, R96370, R96412, R97029, R97266, R97393, R97437, H50949, H51608, H51998, H52116, H57474, H57856, H57995, H59457, H59982, H60111, H63800, H71325, H71371, H79869, H79870, H81092, H81093, H88562, H95676, H95745, N22278, N49708, N52631, N53654, N58430, N68462, N71638, N73373, N78020, N78852, N81176, N94594, W01664, W02147, W02216, W04834, W04843, W17251, W21564, W21539, W23632, W23802, W25419, W32384, W35222, W46230, W46231, W48691, W48692, W52704, W58519, W59976, W69762, W69868, W72449, W72261, W73733, W76298, W76478, W78140, W79490, W80420, W80548, W88575, W93509, W93508, N91306, AA031483, AA031504, AA031462, AA031550, AA034252, AA035471, AA035582, AA037260, AA037507, AA040915, AA039800, AA043301, AA043302, AA043640, AA045108, AA045154, AA054564, AA054624, AA063638, AA062919, AA083744, AA101855, AA102065, AA114883, AA114884, AA114889, AA134060, AA130288, AA157136, AA157191, AA167181, AA196182
666881	<p>Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1503 of SEQ ID NO:65, b is an integer of 15 to 1517, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:65, and where b is greater than or equal to a + 14.</p>	
677071	<p>Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1114 of SEQ ID NO:66, b is an integer of 15 to 1128, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:66, and where b is greater than or equal to a + 14.</p>	R68978, R69696, H04882, N55144

677997	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1014 of SEQ ID NO:67, b is an integer of 15 to 1028, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:67, and where b is greater than or equal to a + 14.	
681507	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2119 of SEQ ID NO:68, b is an integer of 15 to 2133, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:68, and where b is greater than or equal to a + 14.	T54754, T66864, T72618, T72688, T78143, T83143, T98584, R21505, R21607, R31970, R35046, R46863, R53841, R53877, R67660, R79744, R82384, R82702, H03297, H12692, R91706, R94178, R94179, H54137, H75449, H75448, N33743, N35941, N58696, N80900, W04462, W32400, W56025, W56100, W79532, W79612, AA026371, AA026464, AA031315, AA031424, AA080869, AA098956, AA102218, AA100703, AA114096, AA121157, AA122375, AA129155, AA132527, AA132588, AA142892, AA143165, AA190870, AA190963, AA464462
682736	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1622 of SEQ ID NO:69, b is an integer of 15 to 1636, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:69, and where b is greater than or equal to a + 14.	T94338, R08358, T83373, T83518, R19695, R44251, R44251, H23998, H52552, H71674, H72238, N24833, N31760, N39199, N45266, W38403, W60006, W69358, W69359, W86240, W86269, AA057846, AA099877, AA100110, AA114232, AA122230, AA121389, AA121584, AA133105, AA161221, AA173073, AA227447
683116	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1451 of SEQ ID NO:70, b is an integer of 15 to 1465, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:70, and where b is greater than or equal to a + 14.	R36287, H06617, H06748
686494	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1758 of SEQ ID NO:71, b is an integer of 15 to 1772, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:71, and where b is greater than or equal to a + 14.	T62597, T89715, T91664, T93265, T66694, R13787, R16235, R19964, R37645, R40195, R40826, R45066, R46734, R52508, R40195, R40826, R46734, R45066, R61284, H05749, H15041, H15952, H22809, H23014, H23480, H24054, H24162, N51085, N54211, N72577, W02404, AA001565, AA016969
686634	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1149 of SEQ ID NO:72, b is an integer of 15 to	

	1163, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:72, and where b is greater than or equal to a + 14.	
688221	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2908 of SEQ ID NO:73, b is an integer of 15 to 2922, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:73, and where b is greater than or equal to a + 14.	
703498	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1564 of SEQ ID NO:74, b is an integer of 15 to 1578, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:74, and where b is greater than or equal to a + 14.	T52814, T59329, T59725, T63762, T73351, R21181, R22799, R28634, R32514, R32564, R32627, R52152, R53100, H06479, H49626, H49716, H57878, H57879, H88974, H89060, H88974, N20384, N24121, N25447, N26475, N31016, N31420, N31907, N36109, N42690, N44115, N51852, N68213, N70141, N70661, N71996, N72027, N98318, N99378, N99479, W37925, W74077, W79645, AA022687, AA022806, AA055058, AA055059, AA076661, AA079608, AA126936, AA127139, AA128662, AA128648, AA128651, AA128787, AA128816, AA128840, AA132009, AA132016, AA132027, AA132097, AA132119, AA132149, AA133000, AA135495, AA137174, AA146977, AA147156, AA147863, AA147877, AA151371, AA151372, AA151639, AA149559, AA157539, AA158192, AA199713, AA232546
705143	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3219 of SEQ ID NO:75, b is an integer of 15 to 3233, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:75, and where b is greater than or equal to a + 14.	T48699, T48700, T51420, T53379, T54426, T39566, T47698, T49972, T51294, T54475, T53380, T59401, T86970, T91310, T79635, T79720, T83472, T84111, T84891, R33368, R33369, R33998, R36702, R55647, R63964, R64068, R75939, R80444, R80647, H00242, H00243, H01490, H01592, H04172, H04173, H13208, H13574, H21768, H21767, H25031, H26528, H28663, H39497, H39873, H42057, H42102, H44558, H44559, H45520, H45519, R84559, R94554, R94555, R99885, R99886, H79800, H79894, H80460, H81380, N52494, N54896, N57784, N59747, N63528, N68124, N94624, N94848, N98872, W23823, W38568, W49671, W49765, W60785, W60786, W72534, W73483, W73555, W73568, W73487, W76108, W81036, W81073, AA024507.

		AA024592, AA025503, AA025906, AA033919, AA033920, AA034494, AA035699, AA056692, AA058382, AA063569, AA069734, AA069751, AA100815, AA102343, AA116023, AA116024, AA126868, AA127059, AA125936, AA131868, AA131873, AA131977, AA148439, AA148438, AA157513, AA158201, AA158210, AA158926, AA158927, AA458478
705227	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1656 of SEQ ID NO:76, b is an integer of 15 to 1670, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:76, and where b is greater than or equal to a + 14.	
705958	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1163 of SEQ ID NO:77, b is an integer of 15 to 1177, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:77, and where b is greater than or equal to a + 14.	T57095, T57123, T57165, T57188, T57802, T57845, T62006, T62196, T39207, T63715, T64157, T64524, T64552, T65949, T71948, T82364, T82399, T89927, T91496, T91707, T92189, T92222, T92454, T92473, T93065, T93151, T94084, T94162, T94851, T94896, T96577, T98049, T98140, H67037, N69047, N79520, W00695, W16512, N91128
705965	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 815 of SEQ ID NO:78, b is an integer of 15 to 829, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:78, and where b is greater than or equal to a + 14.	AA172120, AA460448
706145	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1129 of SEQ ID NO:79, b is an integer of 15 to 1143, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:79, and where b is greater than or equal to a + 14.	T48474, T54276, T54384, T94387, T94474, R12367, W37454, W40384, W45354, W73244, W74705, W74742, AA100479, AA135190, AA147042, AA149010, AA148966, AA181300, AA187014, AA235328
706473	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1212 of SEQ ID NO:80, b is an integer of 15 to 1226, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:80, and where b is greater than or equal to a + 14.	R40073, N21295, N23386, N24733, N31230, N47403, N47404, N67795, N77874, W03357, AA129311, AA129355, AA133404, AA135135, AA151911, AA161316, AA424925, AA426165
707380	Preferably excluded from the present invention are one or more polynucleotides comprising a	

	nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 560 of SEQ ID NO:81, b is an integer of 15 to 574, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:81, and where b is greater than or equal to a + 14.	
707779	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2029 of SEQ ID NO:82, b is an integer of 15 to 2043, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:82, and where b is greater than or equal to a + 14.	
709441	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1042 of SEQ ID NO:83, b is an integer of 15 to 1056, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:83, and where b is greater than or equal to a + 14.	
710443	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2085 of SEQ ID NO:84, b is an integer of 15 to 2099, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:84, and where b is greater than or equal to a + 14.	T50818, T60229, T64565, T72817, T82382, T74355, R08996, T78357, R14332, R23667, H66532, H66538, N44588, W05229, W52397, W52554, W57898, AA018200, AA057315, AA057333, AA079749, AA081890, AA085154, AA085336, AA102214, AA101505, AA101588, AA151381, AA179541, AA186541, AA190673, AA199674, AA203455, AA224094, AA227755
710603	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3089 of SEQ ID NO:85, b is an integer of 15 to 3103, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:85, and where b is greater than or equal to a + 14.	
710616	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 887 of SEQ ID NO:86, b is an integer of 15 to 901, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:86, and where b is greater than or equal to a + 14.	
710662	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 545 of SEQ ID NO:87, b is an integer of 15 to 559,	H44435, H44469, N91837, N93564, W21040, AA156631, AA169333, AA169539

	where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:87, and where b is greater than or equal to a + 14.	
710917	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2273 of SEQ ID NO:88, b is an integer of 15 to 2287, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:88, and where b is greater than or equal to a + 14.	T69770, T86679, T86771, R07492, T66725, T84963, R00544, R00647, R15450, R23175, R33014, R36435, R79175, R80392, H11173, H16799, H16909, H23735, H23764, H26150, H40756, R96065, H50232, H50267, H81826, H81827, H85100, H89517, H89664, H97062, H97887, N26767, N30263, N41986, N78601, N79449, N79984, N95158, N95364, N99142, W07339, W07819, W21534, W23977, W52960, W56869, W58197, W58306, W72638, W73505, W74799, W77918, W93541, W93542, W96456, W96556, AA009409, AA010115, AA010116, AA010633, AA011223, AA022686, AA022805, AA026132, AA029615, AA044397, AA042848, AA128354, AA128496, AA133694, AA143731, AA159218, AA160796, AA187301, AA187092, AA227584, AA227892
711866	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 593 of SEQ ID NO:89, b is an integer of 15 to 607, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:89, and where b is greater than or equal to a + 14.	N77998
714903	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2324 of SEQ ID NO:90, b is an integer of 15 to 2338, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:90, and where b is greater than or equal to a + 14.	
718139	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1260 of SEQ ID NO:91, b is an integer of 15 to 1274, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:91, and where b is greater than or equal to a + 14.	N23988, N31889, N32345, AA026422, AA026499, AA169674, AA169486
719142	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1397 of SEQ ID NO:92, b is an integer of 15 to 1411, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	

	NO:92. and where b is greater than or equal to a + 14.	
719721	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 715 of SEQ ID NO:93, b is an integer of 15 to 729, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:93, and where b is greater than or equal to a + 14.	
719914	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1781 of SEQ ID NO:94, b is an integer of 15 to 1795, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:94. and where b is greater than or equal to a + 14.	
720134	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 743 of SEQ ID NO:95, b is an integer of 15 to 757, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:95, and where b is greater than or equal to a + 14.	
720270	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 874 of SEQ ID NO:96, b is an integer of 15 to 888, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:96, and where b is greater than or equal to a + 14.	
720583	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2537 of SEQ ID NO:97, b is an integer of 15 to 2551, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:97, and where b is greater than or equal to a + 14.	
720904	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1092 of SEQ ID NO:98, b is an integer of 15 to 1106, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:98. and where b is greater than or equal to a + 14.	
721194	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	



	1254 of SEQ ID NO:99, b is an integer of 15 to 1268, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:99, and where b is greater than or equal to a + 14.	
721271	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1129 of SEQ ID NO:100, b is an integer of 15 to 1143, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:100, and where b is greater than or equal to a + 14.	
723886	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 571 of SEQ ID NO:101, b is an integer of 15 to 585, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:101, and where b is greater than or equal to a + 14.	
723968	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 565 of SEQ ID NO:102, b is an integer of 15 to 579, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:102, and where b is greater than or equal to a + 14.	T49189, T49190, T57872, T57953, T60468, T60622, T68275, T68337, R10623, R10624, H19677, H19678, H25656, H26242, H27936, H27937, H43659, H44727, H45127, R83002, R83052, N44505, N52201, N71160, N75069, N93722, W05541, W05805, W15578, W17186, W21128, W23812, W24230, W25266, W31654, W33175, W37240, W57606, W58199, W58308, W63709, W73015, W73152, W76623, W94088, W94089, W95125, W95242, N90150, AA010262, AA010401, AA010479, AA010507, AA022656, AA022738, AA027253, AA037162, AA037181, AA047030, AA047248, AA045811, AA045906, AA076292, AA076293, AA100771, AA128201, AA129394, AA133794, AA156714, AA167700, AA167814, AA178899, AA178902, AA461557, AA460631, AA428355
725321	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 391 of SEQ ID NO:103, b is an integer of 15 to 405, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:103, and where b is greater than or equal to a + 14.	
725326	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2144 of SEQ ID NO:104, b is an integer of 15 to	

	2158, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:104, and where b is greater than or equal to a + 14.	
726034	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 853 of SEQ ID NO:105, b is an integer of 15 to 867, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:105, and where b is greater than or equal to a + 14.	
726602	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 428 of SEQ ID NO:106, b is an integer of 15 to 442, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:106, and where b is greater than or equal to a + 14.	
726965	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1454 of SEQ ID NO:107, b is an integer of 15 to 1468, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:107, and where b is greater than or equal to a + 14.	
727809	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2474 of SEQ ID NO:108, b is an integer of 15 to 2488, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:108, and where b is greater than or equal to a + 14.	
731703	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1877 of SEQ ID NO:109, b is an integer of 15 to 1891, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:109, and where b is greater than or equal to a + 14.	AA022892, AA046612, AA046520
732840	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1545 of SEQ ID NO:110, b is an integer of 15 to 1559, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:110, and where b is greater than or equal to a + 14.	
733629	Preferably excluded from the present invention are	

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 571 of SEQ ID NO:111, b is an integer of 15 to 585, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:111, and where b is greater than or equal to a + 14.	
733749	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2374 of SEQ ID NO:112, b is an integer of 15 to 2388, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:112, and where b is greater than or equal to a + 14.	R12963, R13257, R14924, R52718, R71280, H26508, H49904, W20330, AA069744, AA112936, AA233733
734119	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2289 of SEQ ID NO:113, b is an integer of 15 to 2303, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:113, and where b is greater than or equal to a + 14.	R68619, R80278, H08190, H08288, N31192, N42303, AA252183
734637	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 737 of SEQ ID NO:114, b is an integer of 15 to 751, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:114, and where b is greater than or equal to a + 14.	N36346, W37360, AA135919, AA188591, AA190956, AA191167
734638	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3089 of SEQ ID NO:115, b is an integer of 15 to 3103, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:115, and where b is greater than or equal to a + 14.	
734865	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 874 of SEQ ID NO:116, b is an integer of 15 to 888, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:116, and where b is greater than or equal to a + 14.	AA126953, AA149347, AA160318, AA173765
738846	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 432 of SEQ ID NO:117, b is an integer of 15 to 446, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:117,	T59751, T60644, T89297, T90329, T90419, H64685, N74880

	and where b is greater than or equal to a + 14.	
740584	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 250 of SEQ ID NO:118, b is an integer of 15 to 264, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:118, and where b is greater than or equal to a + 14.	
741213	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 557 of SEQ ID NO:119, b is an integer of 15 to 571, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:119, and where b is greater than or equal to a + 14.	
741229	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1285 of SEQ ID NO:120, b is an integer of 15 to 1299, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:120, and where b is greater than or equal to a + 14.	
741299	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1635 of SEQ ID NO:121, b is an integer of 15 to 1649, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:121, and where b is greater than or equal to a + 14.	
743134	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2771 of SEQ ID NO:122, b is an integer of 15 to 2785, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:122, and where b is greater than or equal to a + 14.	
744680	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1954 of SEQ ID NO:123, b is an integer of 15 to 1968, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:123, and where b is greater than or equal to a + 14.	
744705	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	R87645, N24592, N24957, N25844, N30699, N33738, N41313, W61296, W65392, AA029407, AA029406, AA037259, AA199742, AA199790

	1691 of SEQ ID NO:124, b is an integer of 15 to 1705, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:124, and where b is greater than or equal to a + 14.	
745337	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2367 of SEQ ID NO:125, b is an integer of 15 to 2381, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:125, and where b is greater than or equal to a + 14.	T40398, T41245, T26513, T26514, T26515, R50522, R51530, R51531, R52099, R52195, H15883, H40745, H45530, H45531, H49233, H49234, H51160, H61211, H62117, H93148, N29189, N48350, N49126, N56774, N66819, N71954, N99689, W48612, W67303, W67419, AA025497, AA040666, AA046266, AA046345, AA053465, AA053234, AA074556, AA074528, AA075941, AA079527, AA084953, AA132753, AA132802, AA159029, AA159030, AA159169, AA159274, AA160752, AA262679, AA427433, AA427688
745570	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1699 of SEQ ID NO:126, b is an integer of 15 to 1713, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:126, and where b is greater than or equal to a + 14.	T55379, T78988, T95100, R23674, R61397, R61442, R73724, R75646, R75752, R79492, N59852, N67315, N70855, W06865, W24675, W78989, W78873, W80595, W94313, AA031905, AA032006, AA042987, AA043041, AA081690, AA460259, AA463823
746078	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1500 of SEQ ID NO:127, b is an integer of 15 to 1514, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:127, and where b is greater than or equal to a + 14.	
750595	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2035 of SEQ ID NO:128, b is an integer of 15 to 2049, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:128, and where b is greater than or equal to a + 14.	R38607, R46103, R46103, R55827, R56073, H05009, H05010, H05764, H05871, H70717, N22562, N50960, N66199, N75783, W32323, W32654, AA115520, AA114922, AA161333, AA253251, AA460716, AA460890
750633	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1252 of SEQ ID NO:129, b is an integer of 15 to 1266, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:129, and where b is greater than or equal to a + 14.	
750766	Preferably excluded from the present invention are	T61022, T61574, N74315, N98348,

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1081 of SEQ ID NO:130, b is an integer of 15 to 1095, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:130, and where b is greater than or equal to a + 14.	N98517, W05223, W31082, W42972, W42973, W69783, W69731, AA029869, AA029316, AA040718, AA040719, AA148660, AA148661, AA150953, AA151043, AA181851, AA186586, AA197153, AA197137, AA463512
752225	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2876 of SEQ ID NO:131, b is an integer of 15 to 2890, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:131, and where b is greater than or equal to a + 14.	
754538	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 553 of SEQ ID NO:132, b is an integer of 15 to 567, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:132, and where b is greater than or equal to a + 14.	
754820	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 772 of SEQ ID NO:133, b is an integer of 15 to 786, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:133, and where b is greater than or equal to a + 14.	
756565	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1207 of SEQ ID NO:134, b is an integer of 15 to 1221, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:134, and where b is greater than or equal to a + 14.	R19918, R23647, N50210, W31589, W81698, AA172287, AA173866
756793	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1907 of SEQ ID NO:135, b is an integer of 15 to 1921, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:135, and where b is greater than or equal to a + 14.	
757431	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 989 of SEQ ID NO:136, b is an integer of 15 to 1003, where both a and b correspond to the	T61971, R11782, R51982, R51981, H24532, H42326, H42923, H38029, H95132, N77240, N78638, N81066, W07134, W16665, W19796, W42604, W42607, W46765, W67123, W68839, W69749, W69856, AA019415,

	positions of nucleotide residues shown in SEQ ID NO:136, and where b is greater than or equal to a + 14.	AA039588, AA039589, AA055454, AA101116, AA129779, AA148487, AA148486, AA188297, AA251345, AA251440, AA430079
757478	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 864 of SEQ ID NO:137, b is an integer of 15 to 878, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:137, and where b is greater than or equal to a + 14.	
757695	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2491 of SEQ ID NO:138, b is an integer of 15 to 2505, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:138, and where b is greater than or equal to a + 14.	
760876	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 258 of SEQ ID NO:139, b is an integer of 15 to 272, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:139, and where b is greater than or equal to a + 14.	
761528	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1578 of SEQ ID NO:140, b is an integer of 15 to 1592, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:140, and where b is greater than or equal to a + 14.	T61999, T62191, N57708, N59676, AA001349, AA001982, AA464252, AA464360
761936	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 828 of SEQ ID NO:141, b is an integer of 15 to 842, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:141, and where b is greater than or equal to a + 14.	
761944	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3189 of SEQ ID NO:142, b is an integer of 15 to 3203, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:142, and where b is greater than or equal to a + 14.	
764913	Preferably excluded from the present invention are one or more polynucleotides comprising a	

	nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3460 of SEQ ID NO:143, b is an integer of 15 to 3474, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:143, and where b is greater than or equal to a + 14.	
764941	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3269 of SEQ ID NO:144, b is an integer of 15 to 3283, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:144, and where b is greater than or equal to a + 14.	R23682, R25571, R40976, R44135, R40976, R44135, R62882, R63815, R64423, H05633, H12992, H13863, H13918, H19024, H19316, H22903, H24291, H25927, H27667, H27668, H28317, H28318, H39907, R85243, R93863, R98771, R98772, H57201, N27777, N62312, N65975, N79067, N94237, N98931, W19838, W24064, W31159, W40175, W56611, W60342, W81204, W81206, W81207, W93359, W93447, AA037481, AA121685, AA199815, AA199905, AA224144, AA224298, AA226800, AA233529, AA460353, AA419114, AA419149
765903	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1804 of SEQ ID NO:145, b is an integer of 15 to 1818, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:145, and where b is greater than or equal to a + 14.	R13305, R40788, R50797, R50915, R40788, R56524, R59696, R60018, H09347, H09407, H19221, H51737, H73641, H73642, H90518, H90614, N44279, N45966, N51825, N52967, W15394, W58520, W78069, AA040801, AA041239, AA193309, AA194263, AA425331, AA428477
766122	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 500 of SEQ ID NO:146, b is an integer of 15 to 514, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:146, and where b is greater than or equal to a + 14.	
766719	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2521 of SEQ ID NO:147, b is an integer of 15 to 2535, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:147, and where b is greater than or equal to a + 14.	T61333, T52655, T69392, T70444, T72342, T72383, T92195, T94967, T87463, R11623, R12048, T78115, T78851, T79946, T83322, T96968, T97079, T97411, R18454, R21014, R21442, R23107, R23108, R23221, R35121, R37110, R38010, R38400, R41312, R49509, R52450, R41312, R49509, R56650, R56651, R63015, R66787, R68818, R68926, R69892, R74178, R74263, R77115, R77116, R79241, H00340, H00401, H02817, H02818, H03234, H12313, H12366, H21082, H21083, H21575, H39686, H43389, H45308, H47274, H47506, R85441, R89072, R89172, R91233, R92017, R92062, R93635, R93790, R97298, R97323, H63388, H63440,



		H67848, H68634, H70208, H73093, H73906, H74098, H73454, H77851, H77903, H89736, H89799, H96012, H96999, H97208, H97750, H98624, N25144, N40991, N41793, N45001, N53013, N63728, N66887, N67316, N76307, N92620, N93546, N98219, N99033, W01073, W20158, W21151, W42485, W42532, W44556, W44547, W46274, W46275, W46293, W46760, W46907, W53017, W57643, W74262, W79876, AA028145, AA028182, AA028896, AA037086, AA043121, AA043969, AA190453, AA191396, AA193355, AA194390, AA194476, AA461330
767655	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2301 of SEQ ID NO:148, b is an integer of 15 to 2315, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:148, and where b is greater than or equal to a + 14.	
767941	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2590 of SEQ ID NO:149, b is an integer of 15 to 2604, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:149, and where b is greater than or equal to a + 14.	
768035	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 671 of SEQ ID NO:150, b is an integer of 15 to 685, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:150, and where b is greater than or equal to a + 14.	R14455, R69813, N57212
769888	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1089 of SEQ ID NO:151, b is an integer of 15 to 1103, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:151, and where b is greater than or equal to a + 14.	
771671	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1103 of SEQ ID NO:152, b is an integer of 15 to 1117, where both a and b correspond to the	

	positions of nucleotide residues shown in SEQ ID NO:152, and where b is greater than or equal to a + 14.	
772876	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2024 of SEQ ID NO:153, b is an integer of 15 to 2038, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:153, and where b is greater than or equal to a + 14.	H48052, H70779, H70778, W55869, AA024474, AA128713, AA158771
773150	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 631 of SEQ ID NO:154, b is an integer of 15 to 645, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:154, and where b is greater than or equal to a + 14.	
773398	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1582 of SEQ ID NO:155, b is an integer of 15 to 1596, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:155, and where b is greater than or equal to a + 14.	W63659, AA080975, AA121709, AA126836, AA127584, AA134406, AA147005, AA160159, AA164695, AA165611, AA171799, AA233065
773647	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1640 of SEQ ID NO:156, b is an integer of 15 to 1654, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:156, and where b is greater than or equal to a + 14.	
773927	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1801 of SEQ ID NO:157, b is an integer of 15 to 1815, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:157, and where b is greater than or equal to a + 14.	
774100	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1383 of SEQ ID NO:158, b is an integer of 15 to 1397, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:158, and where b is greater than or equal to a + 14.	T90757, R17008, R18310, R98163, N94386, W21282, W55964, AA037125, AA081882, AA135947, AA136510, AA155940
774101	Preferably excluded from the present invention are	R39231, R42286, R42286, H21387,

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 942 of SEQ ID NO:159, b is an integer of 15 to 956, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:159, and where b is greater than or equal to a + 14.	H21430, H23195, N62733, N66184, N78457, N80264, N99178, AA045207, AA133141, AA135395, AA135659, AA135672, AA165439, AA165438, AA418539
774159	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2251 of SEQ ID NO:160, b is an integer of 15 to 2265, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:160, and where b is greater than or equal to a + 14.	
774341	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 984 of SEQ ID NO:161, b is an integer of 15 to 998, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:161, and where b is greater than or equal to a + 14.	
774371	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1736 of SEQ ID NO:162, b is an integer of 15 to 1750, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:162, and where b is greater than or equal to a + 14.	
777534	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3082 of SEQ ID NO:163, b is an integer of 15 to 3096, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:163, and where b is greater than or equal to a + 14.	T64606, T66246, R11978, R00627, R13377, R14039, R56458, R60447, R62230, R64606, R72959, R73576, R81926, H29425, H60246, H85563, N29906, N36864, N42958, N46134, W15640, W39775, N90323, AA007317, AA021093, AA026823, AA026955, AA034103, AA064957, AA075106, AA113409, AA134171, AA134170, AA136815, AA159636
777623	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1202 of SEQ ID NO:164, b is an integer of 15 to 1216, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:164, and where b is greater than or equal to a + 14.	
779194	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 766 of SEQ ID NO:165, b is an integer of 15 to	

	780, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:165, and where b is greater than or equal to a + 14.	
779387	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3366 of SEQ ID NO:166, b is an integer of 15 to 3380, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:166, and where b is greater than or equal to a + 14.	T47850, T70030, T70097, T97303, T97417, R26372, R27996, R28099, R48027, R76100, R76151, R78969, H01122, H01123, H69526, H69981, H88816, H88817, H88817, H99882, N28568, N36017, N36801, N45965, W39683, W44987, W46393, W47394, W47424, W52700, AA035254, AA035500, AA035102, AA039488, AA039489, AA069554, AA099394, AA099515, AA130160, AA147727, AA157616, AA182043, AA190771, AA191381, AA232291, AA233456, AA233496, AA234039, AA236285, AA427888, AA427971
779790	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1631 of SEQ ID NO:167, b is an integer of 15 to 1645, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:167, and where b is greater than or equal to a + 14.	
779818	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1134 of SEQ ID NO:168, b is an integer of 15 to 1148, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:168, and where b is greater than or equal to a + 14.	T80350, AA172153
779819	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2049 of SEQ ID NO:169, b is an integer of 15 to 2063, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:169, and where b is greater than or equal to a + 14.	T53889, T54036, T56942, T56943, T64670, T67876, T68027, T69603, T69675, T72245, R13895, R37729, R39041, R66644, R67363, H61695, H61696, H79871, H79872, H94755, N54393, N70849, N76357, W06839, W67873, W67874, W94734, AA036740, AA041382, AA062758, AA171946, AA464456
780634	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2902 of SEQ ID NO:170, b is an integer of 15 to 2916, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:170, and where b is greater than or equal to a + 14.	T91261, R37843, R37928, R51931, R74365, R74367, H00839, H01223, N66989, W31896, W39259, AA251009, AA251479, AA262830, AA418381, AA418534
780638	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	

	formula of a-b, where a is any integer between 1 to 2515 of SEQ ID NO:171, b is an integer of 15 to 2529, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:171, and where b is greater than or equal to a + 14.	
780773	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 797 of SEQ ID NO:172, b is an integer of 15 to 811, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:172, and where b is greater than or equal to a + 14.	T94203, R26771, R31421, R31436, R37199, R77716, R77727, R78060, R79203, R79205, R79469, R79472, H20928, H43091, H44086, H44174, N27827, N48150, N48678, N49710, N49816, N53296, N72238, N92833, W32965, W75970, AA046240, AA122068, AA182933
780778	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2207 of SEQ ID NO:173, b is an integer of 15 to 2221, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:173, and where b is greater than or equal to a + 14.	T93197, R63521, R63567, H03648, H03649, H63542, H63585, H71373, H84876, H85286, H87956, H88009, H88232, H88344, N24216, N25549, N34146, W85792, AA084961, AA084960, AA088785, AA100682
780873	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 743 of SEQ ID NO:174, b is an integer of 15 to 757, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:174, and where b is greater than or equal to a + 14.	T80944, R64627, R64628, R70696, R70697, R71119, R71300, H03131, R94989, R98519, R98545, H60772, H82562, H89214, N31387, N70670, N77981, W58445, W58480, AA046891, AA046892, AA465001
782113	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2207 of SEQ ID NO:175, b is an integer of 15 to 2221, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:175, and where b is greater than or equal to a + 14.	
782153	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1499 of SEQ ID NO:176, b is an integer of 15 to 1513, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:176, and where b is greater than or equal to a + 14.	T73302, T73365, R24876, R36231, R36232, R68235, R68528, R73630, R75759, H28780, H28756, H69128, H69129, N22916, N31304, N38744, N48381, W02714, W15469, W25572, W37894, W37954, W39623, W60018, AA026689, AA026700, AA187498, AA188108, AA187980, AA261951
782376	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4069 of SEQ ID NO:177, b is an integer of 15 to 4083, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:177, and where b is greater than or equal to a + 14.	

	14.	
782420	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2718 of SEQ ID NO:178, b is an integer of 15 to 2732, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:178, and where b is greater than or equal to a + 14.	
782672	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 858 of SEQ ID NO:179, b is an integer of 15 to 872, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:179, and where b is greater than or equal to a + 14.	R33945, H97542, W93819, AA227573, AA227882, AA460150, AA460748
783148	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2237 of SEQ ID NO:180, b is an integer of 15 to 2251, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:180, and where b is greater than or equal to a + 14.	
783510	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2775 of SEQ ID NO:181, b is an integer of 15 to 2789, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:181, and where b is greater than or equal to a + 14.	
783734	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3503 of SEQ ID NO:182, b is an integer of 15 to 3517, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:182, and where b is greater than or equal to a + 14.	
784201	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 844 of SEQ ID NO:183, b is an integer of 15 to 858, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:183, and where b is greater than or equal to a + 14.	
784381	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	

	2373 of SEQ ID NO:184, b is an integer of 15 to 2387, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:184, and where b is greater than or equal to a + 14.	
784387	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2871 of SEQ ID NO:185, b is an integer of 15 to 2885, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:185, and where b is greater than or equal to a + 14.	R32962, R65669, R70746, R80363, H06027, H11579, H84180, AA010747, AA074888, AA223293
784639	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2164 of SEQ ID NO:186, b is an integer of 15 to 2178, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:186, and where b is greater than or equal to a + 14.	
784641	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1240 of SEQ ID NO:187, b is an integer of 15 to 1254, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:187, and where b is greater than or equal to a + 14.	
785053	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1465 of SEQ ID NO:188, b is an integer of 15 to 1479, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:188, and where b is greater than or equal to a + 14.	
785142	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3397 of SEQ ID NO:189, b is an integer of 15 to 3411, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:189, and where b is greater than or equal to a + 14.	
785584	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2603 of SEQ ID NO:190, b is an integer of 15 to 2617, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	

	NO:190, and where b is greater than or equal to a + 14.	
785795	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3130 of SEQ ID NO:191, b is an integer of 15 to 3144, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:191, and where b is greater than or equal to a + 14.	
786283	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2556 of SEQ ID NO:192, b is an integer of 15 to 2570, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:192, and where b is greater than or equal to a + 14.	
786335	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1510 of SEQ ID NO:193, b is an integer of 15 to 1524, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:193, and where b is greater than or equal to a + 14.	
786511	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1664 of SEQ ID NO:194, b is an integer of 15 to 1678, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:194, and where b is greater than or equal to a + 14.	
787330	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2810 of SEQ ID NO:195, b is an integer of 15 to 2824, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:195, and where b is greater than or equal to a + 14.	
787377	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4246 of SEQ ID NO:196, b is an integer of 15 to 4260, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:196, and where b is greater than or equal to a + 14.	T56159, T63176, T65462, T87530, T87531, T90458, T83039, T83977, T84180, R13022, H23316, H40518, R88510, R91571, H62310, H73670, H73413, H77723, H81660, N32679, N44824, N50589, N55310, N64096, N69880, N92004, N99388, W90426, W90148, AA151077, AA179970, AA180462, AA195170, AA195270, AA195707, AA195744, AA425027



787662	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3103 of SEQ ID NO:197, b is an integer of 15 to 3117, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:197, and where b is greater than or equal to a + 14.	
788754	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2469 of SEQ ID NO:198, b is an integer of 15 to 2483, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:198, and where b is greater than or equal to a + 14.	
789351	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1224 of SEQ ID NO:199, b is an integer of 15 to 1238, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:199, and where b is greater than or equal to a + 14.	
789466	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 626 of SEQ ID NO:200, b is an integer of 15 to 640, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:200, and where b is greater than or equal to a + 14.	N62718, AA211883, AA252981
790396	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1425 of SEQ ID NO:201, b is an integer of 15 to 1439, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:201, and where b is greater than or equal to a + 14.	
791673	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1233 of SEQ ID NO:202, b is an integer of 15 to 1247, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:202, and where b is greater than or equal to a + 14.	
792080	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	T80259, N44613

	732 of SEQ ID NO:203, b is an integer of 15 to 746, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:203, and where b is greater than or equal to a + 14.	
793025	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2156 of SEQ ID NO:204, b is an integer of 15 to 2170, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:204, and where b is greater than or equal to a + 14.	
793043	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2606 of SEQ ID NO:205, b is an integer of 15 to 2620, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:205, and where b is greater than or equal to a + 14.	
793386	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1000 of SEQ ID NO:206, b is an integer of 15 to 1014, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:206, and where b is greater than or equal to a + 14.	
795144	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1353 of SEQ ID NO:207, b is an integer of 15 to 1367, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:207, and where b is greater than or equal to a + 14.	
795911	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1484 of SEQ ID NO:208, b is an integer of 15 to 1498, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:208, and where b is greater than or equal to a + 14.	T40041, R75763, H07057, H43863, H53392, H71544, H84663, N28804, N94279, W19740, AA017623, AA057111, AA058918, AA195576
795962	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2351 of SEQ ID NO:209, b is an integer of 15 to 2365, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:209, and where b is greater than or equal to a +	

	14.	
796221	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 996 of SEQ ID NO:210, b is an integer of 15 to 1010, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:210, and where b is greater than or equal to a + 14.	
796283	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1534 of SEQ ID NO:211, b is an integer of 15 to 1548, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:211, and where b is greater than or equal to a + 14.	
796392	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1515 of SEQ ID NO:212, b is an integer of 15 to 1529, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:212, and where b is greater than or equal to a + 14.	
797655	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2561 of SEQ ID NO:213, b is an integer of 15 to 2575, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:213, and where b is greater than or equal to a + 14.	
799486	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2026 of SEQ ID NO:214, b is an integer of 15 to 2040, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:214, and where b is greater than or equal to a + 14.	
799681	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 310 of SEQ ID NO:215, b is an integer of 15 to 324, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:215, and where b is greater than or equal to a + 14.	
800221	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	

	formula of a-b, where a is any integer between 1 to 1461 of SEQ ID NO:216, b is an integer of 15 to 1475, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:216, and where b is greater than or equal to a + 14.	
800376	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1373 of SEQ ID NO:217, b is an integer of 15 to 1387, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:217, and where b is greater than or equal to a + 14.	
800567	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1819 of SEQ ID NO:218, b is an integer of 15 to 1833, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:218, and where b is greater than or equal to a + 14.	
800652	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2578 of SEQ ID NO:219, b is an integer of 15 to 2592, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:219, and where b is greater than or equal to a + 14.	
800748	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2390 of SEQ ID NO:220, b is an integer of 15 to 2404, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:220, and where b is greater than or equal to a + 14.	
802032	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2656 of SEQ ID NO:221, b is an integer of 15 to 2670, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:221, and where b is greater than or equal to a + 14.	
802050	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1742 of SEQ ID NO:222, b is an integer of 15 to 1756, where both a and b correspond to the	T53455, T53456, T64307, T64694, T71011, T71073, R09951, R12591, R18956, R22898, R23000, R24702, R25196, R27761, R27844, R43964, R46320, R54857, R54866, R43964, R60214, R60448, R69089, R69203.

	positions of nucleotide residues shown in SEQ ID NO:222, and where b is greater than or equal to a + 14.	R70698, R70699, R71940, R72061, R73121, R73174, H22669, H22686, H25678, H27383, H27962, H27963, H28646, H29871, H29967, H39890, H37770, H44247, H44701, H45421, R83508, R83540, R88432, H65089, H65136, H82626, H82627, H83486, H83487, H84231, H84553, H85813, H86051, H86914, H86915, H87763, H87812, H92048, H92218, H92310, H92366, H93438, H94153, H94253, H95552, H96048, H96410, N78955, N80229, N95017, N99227, W17349, W23863, W37217, W37556, W38519, AA000983, AA000984, AA001124, AA001178, AA012816, AA012817, AA012821, AA012837, AA013129, AA013296, AA013322, AA015870, AA016173, AA016172, AA016244, AA017427, AA017532, AA017510, AA017650, AA018432, AA018433, AA018668, AA018669, AA018788, AA018789, AA018932, AA018933, AA018800, AA019295, AA019296, AA019616, AA019634, AA019820, AA021468, AA021467, AA021555, AA020761, AA036874, AA040654, AA046028, AA046080, AA047875, AA054063, AA054205, AA053997, AA058827, AA058954, AA059096, AA059097, AA121321, AA121453, AA425728, AA427798
805551	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2365 of SEQ ID NO:223, b is an integer of 15 to 2379, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:223, and where b is greater than or equal to a + 14.	
805662	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2497 of SEQ ID NO:224, b is an integer of 15 to 2511, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:224, and where b is greater than or equal to a + 14.	
805750	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 587 of SEQ ID NO:225, b is an integer of 15 to 601, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:225,	

	and where b is greater than or equal to a + 14.	
805860	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 493 of SEQ ID NO:226, b is an integer of 15 to 507, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:226, and where b is greater than or equal to a + 14.	
805886	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1027 of SEQ ID NO:227, b is an integer of 15 to 1041, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:227, and where b is greater than or equal to a + 14.	
806706	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1644 of SEQ ID NO:228, b is an integer of 15 to 1658, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:228, and where b is greater than or equal to a + 14.	
811637	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1602 of SEQ ID NO:229, b is an integer of 15 to 1616, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:229, and where b is greater than or equal to a + 14.	T59548, T59503, T61640, R47891, R53382, R69888, R78209, H11069, H49549, H70903, H70985, H85798, H88010, H88233, H89244, H70903, N48638, N67235, W16765, W44942, W72288, W76314, AA010071, AA013237, AA013331, AA018680, AA102724, AA132323, AA143684, AA173703, AA213813, AA213892, AA214580, AA223769, AA256832
811782	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1914 of SEQ ID NO:230, b is an integer of 15 to 1928, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:230, and where b is greater than or equal to a + 14.	
812338	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1221 of SEQ ID NO:231, b is an integer of 15 to 1235, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:231, and where b is greater than or equal to a + 14.	R14488, H20945, H85272, N36641, W52527
812439	Preferably excluded from the present invention are one or more polynucleotides comprising a	

	nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2533 of SEQ ID NO:232, b is an integer of 15 to 2547, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:232, and where b is greater than or equal to a + 14.	
812645	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 990 of SEQ ID NO:233, b is an integer of 15 to 1004, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:233, and where b is greater than or equal to a + 14.	T47861, T56070, T51490, T53266, T56036, T62925, T63268, T63920, T65968, T90499, T92798, T94959, T95012, R62607, R62656, R72831, R73017, R73018, R73285, H03018, H04300, H25930, H25956, H26558, H28208, H43607, H44636, H44649, H45454, H45544, R92028, R97989, N49912, N55253, N76103, N76860, N91838, W23759, W73482, W94362, W94471, N89886, AA046695, AA187153, AA187175
812770	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2096 of SEQ ID NO:234, b is an integer of 15 to 2110, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:234, and where b is greater than or equal to a + 14.	T39754, T39831, T39352, T62702, T92238, T92280, T92968, T94256, T86698, T86793, T88969, R08026, R09266, T99878, R18049, R19212, R21573, R21599, R65715, H21005, H25255, H48018, R83571, H51996, H62745, H63322, H71550, H73155, H73631, H78047, H78489, H85242, H84914, H95643, N25403, N25830, N39488, N44902, N45050, N72391, N79701, N94855, W02622, W03117, W04789, W30780, W31119, W31146, W44536, W57915, W67327, W68227, W72924, W79796, W94218, W92184, AA029874, AA041265, AA070556, AA070987, AA071217, AA075110, AA075613, AA084227, AA086143, AA126166, AA127661, AA135577, AA135557, AA135653, AA136492, AA172281, AA180259, AA180507, AA192889, AA211798, AA427386
812893	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3514 of SEQ ID NO:235, b is an integer of 15 to 3528, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:235, and where b is greater than or equal to a + 14.	
813080	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 524 of SEQ ID NO:236, b is an integer of 15 to 538, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:236, and where b is greater than or equal to a + 14.	

813139	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2014 of SEQ ID NO:237, b is an integer of 15 to 2028, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:237, and where b is greater than or equal to a + 14.	T80022, T80132, H57912, H61357
815326	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1501 of SEQ ID NO:238, b is an integer of 15 to 1515, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:238, and where b is greater than or equal to a + 14.	
815740	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1714 of SEQ ID NO:239, b is an integer of 15 to 1728, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:239, and where b is greater than or equal to a + 14.	T48264, T58091, T94419, T94506, H21757, H99081, N26947, N36795, N45955, N66570, N70184, N98830, W32113, W32171, W55906, W55927, W61339, W60040, W80432, W80562, W81101, AA025239, AA026026, AA046321, AA046146, AA204703
815812	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1103 of SEQ ID NO:240, b is an integer of 15 to 1117, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:240, and where b is greater than or equal to a + 14.	T90521, R07475, R07526, T98792, T98793, R45273, R52676, R45273, R59860, R61768, H07859, H08666, H38684, R83910, R83909, H59038, H59037, H63995, H64043, H67088, H67624, N26301, N32125, N33562, N41543, N56792, AA010140, AA010139, AA011662, AA011709, AA044827, AA129084, AA151918, AA173794
824865	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2357 of SEQ ID NO:241, b is an integer of 15 to 2371, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:241, and where b is greater than or equal to a + 14.	T49439, T49440, T74012, R05533, R05643, R71805, R79363, R79364, R91535, H61048, H61610, H66233, H69618, H70463, H70613, H70890, H71293, H78547, H81120, H91295, H91390, N57962, N64309, N70328, N71483, N74460, N99513, W02797, W03055, N91438, AA022995, AA022463, AA151573, AA151722
825138	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3262 of SEQ ID NO:242, b is an integer of 15 to 3276, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:242, and where b is greater than or equal to a + 14.	
825535	Preferably excluded from the present invention are one or more polynucleotides comprising a	



	nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 722 of SEQ ID NO:243, b is an integer of 15 to 736, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:243, and where b is greater than or equal to a + 14.	
826203	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2297 of SEQ ID NO:244, b is an integer of 15 to 2311, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:244, and where b is greater than or equal to a + 14.	
827046	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4051 of SEQ ID NO:245, b is an integer of 15 to 4065, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:245, and where b is greater than or equal to a + 14.	T63435, T63759, T87711, T94058, T94805, T94844, T94889, T86880, T86881, T80042, R00243, R00352, R13034, R31385, R32380, R32381, R38760, R40284, R40418, R51387, R51490, R40284, R40418, R71495, R71549, R77110, R77111, R81535, R81534, H02414, H04748, H04837, H05744, H05850, H12437, H12438, H24410, H84506, H88056, H88079, H88080, H88266, H88520, H88523, H88561, H88567, H88811, H89242, H89243, H89340, H89522, H88080, H88520, H88811, H89243, H89340, N22133, N30400, N47068, N50832, N62530, N63385, N66711, N66730, N66728, N67341, N67944, N71637, N71853, N71904, N79428, N66391, W01663, W25692, W56009, W56318, AA055188, AA055919, AA076369, AA076498, AA079647, AA079553, AA115543, AA115054, AA122371, AA121426, AA164879, AA164878, AA173652, AA173651, AA258324, AA258532, AA460643, AA460990, AA280874, AA280953, AA525305, AA525328, AA526641, AA552970, AA593665, AA570032, AA570417, AA572744, AA728803, AA728824, AA746917
827168	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1471 of SEQ ID NO:246, b is an integer of 15 to 1485, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:246, and where b is greater than or equal to a + 14.	
827195	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	T77108, R11986

	formula of a-b, where a is any integer between 1 to 1472 of SEQ ID NO:247, b is an integer of 15 to 1486, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:247, and where b is greater than or equal to a + 14.	
827249	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1980 of SEQ ID NO:248, b is an integer of 15 to 1994, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:248, and where b is greater than or equal to a + 14.	
827447	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1647 of SEQ ID NO:249, b is an integer of 15 to 1661, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:249, and where b is greater than or equal to a + 14.	
827515	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2344 of SEQ ID NO:250, b is an integer of 15 to 2358, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:250, and where b is greater than or equal to a + 14.	
827621	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 683 of SEQ ID NO:251, b is an integer of 15 to 697, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:251, and where b is greater than or equal to a + 14.	
827883	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2944 of SEQ ID NO:252, b is an integer of 15 to 2958, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:252, and where b is greater than or equal to a + 14.	
828040	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2513 of SEQ ID NO:253, b is an integer of 15 to 2527, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	T78257, T81592, T83408, T98857, T99453, R24503, R24602, R34836, R34932, R52437, R52501, R52500, H10205, H12773, H12827, H20540, H20632, H40525, R92740, H51257, H51827, H78114, H78113, H93565, N30243, N30795, N41570, N57137,

	NO:253. and where b is greater than or equal to a + 14.	N67626, N71081, N75985, N76340, W01320, W04402, W04410, W52430, W52471, W61358, W60048, AA040527, AA040528, AA044739, AA044794, AA133224, AA130908, AA130822, AA142867, AA151675, AA151755, AA470461, AA524808, AA715484, AA720952, AA730460, AA912077, AA961362, AA961363
828360	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1169 of SEQ ID NO:254, b is an integer of 15 to 1183, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:254, and where b is greater than or equal to a + 14.	T39184, T40461, T63411, T71316, T89154, T89248, R21853, R21870, R22009, R22010, R33088, R33178, R51751, R66596, R70491, R70581, R82639, R82684, H00709, H11650, H17014, H19338, H38138, H79933, H80766, H82262, H83318, H83679, N22884, N33281, N34791, N44515, N52015, N54628, N64069, N64136, N74874, N74875, N92414, N92810, W01786, W05708, W19189, W19348, W24929, W42669, W45192, W46546, W47392, W47422, W60251, W67177, W67178, W79541, W79622, W84535, W90125, W94769, W95205, AA027281, AA035333, AA035334, AA037062, AA041467, AA043350, AA044657, AA056021, AA056069, AA056757, AA058356, AA058435, AA084638, AA126486, AA126612, AA127391, AA127516, AA127573, AA127613, AA133186, AA133373, AA133313, AA131305, AA131548, AA134952, AA134902, AA148589, AA159517, AA172259, AA210910, AA210909
828506	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2037 of SEQ ID NO:255, b is an integer of 15 to 2051, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:255, and where b is greater than or equal to a + 14.	T46854, T51134, T63778, T63812, T70197, T94033, T94784, R11818, T78022, T78185, T97720, T97823, T99847, R00419, R06015, R14242, R15768, R16272, R22870, R24857, R24859, R36940, R37122, R38128, R40537, R40537, R64393, R66369, R66406, R67096, R67139, R69618, R69710, R77468, R81558, R82623, H03456, H03540, H03681, H04772, H04868, H27957, R83222, R97633, R99738, H54239, H71042, H75761, H75896, H77581, H83096, H83756, H87347, H87884, H90290, H90942, H94688, H95048, H99140, N21141, N23049, N24389, N24821, N25733, N28713, N33386, N35058, N35850, N36275, N36446, N36851, N41970, N45071, N46122, N46168, N47026, N66222, N66465, N71931, N74548, N99686, W02943, W37106, W37807, W39258, W40485, W52921, W56441,

		W57563, W57776, W58586, W58587, W79329, W92948, W92949, AA011548, AA011527, AA023000, AA022467, AA024997, AA025209, AA025216, AA025227, AA025990, AA028003, AA033608, AA037401, AA039442, AA039443, AA043901, AA056925, AA057070, AA074081, AA083873, AA099028, AA112887, AA115001, AA114964, AA128132, AA125887, AA135310, AA136391, AA148227, AA149076, AA149077, AA148545, AA156636, AA160112, AA160113, AA169459, AA181734, AA187469, AA187626, AA189064, AA191260, AA191538, AA207172, AA207171, AA224359, AA226905, AA226915, AA235559, AA459908
828517	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 672 of SEQ ID NO:256, b is an integer of 15 to 686, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:256, and where b is greater than or equal to a + 14.	H15804, H18452, AA146592, AA149939, AA149892, AA160732, AA191608, AA548983, AA554733, AA600759, AA865400, AA907885, AA954237
828898	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2308 of SEQ ID NO:257, b is an integer of 15 to 2322, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:257, and where b is greater than or equal to a + 14.	T61075, T91806, R09240, R09355, T99060, T99658, R07031, R07043, R07075, R07102, R28642, R32575, R36973, R47863, R47864, H15908, H16014, H19893, H40060, H44782, R92942, R92943, H59884, H59885, H67802, H68075, N58069, N64287, N71857, N72594, N98414, W02374, W30702, W31135, W37192, W60618, AA045338, AA055209, AA055299, AA114871, AA114872, AA120998, AA122326, AA121537, AA127701, AA156728, AA181228, AA181833, AA192414, AA192434, AA468743, AA468763, AA523325, AA523458, AA526457, AA533984, F16728, AA584430, AA601952, AA613639, AA570586, AA665254, AA728791, AA728810, AA729554, AA729948, AA736948, AA827503, AA863088, AA865405, AA872707, AA873211, AA877782, AA879306, AA886586, AA894507, AA908568, AA919045, AA939288, AA960779, A1053399, A1053494, A1053497, A1053505, A1053537, A1053548, A1053565, A1053578, A1053607, A1053632, A1053648, A1053687, A1053698, A1053723, A1053746, A1053750, A1053867, A1053875, A1053904, A1053976, A1054008, A1054040,

		AI054099, AI054098, AI054186, AI054207, AI054201, AI054218, AI054230, AI054262, AI054282, AI054295, AI054324, AI054347, AI054401, F18087, W92362, AA093600
828959	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2247 of SEQ ID NO:258, b is an integer of 15 to 2261, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:258, and where b is greater than or equal to a + 14.	T49110, T49111, H03714, H45126, H45429, H88387, H88456, H88387, N20209, N29249, N29276, N32771, N36721, N41772, N41777, N79003, N92380, W15282, W19718, W24622, W58051, W58374, N89975, AA029669, AA181566, AA182461, AA186831, AA186832, AA460703, AA460878, AA430595, AA430596, AA430747, AA557632, AA610311, AA687639, AA872173, AA887603, AA992459, N83579, AA642545, C20993, AA091179
829081	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1360 of SEQ ID NO:259, b is an integer of 15 to 1374, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:259, and where b is greater than or equal to a + 14.	
830069	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1944 of SEQ ID NO:260, b is an integer of 15 to 1958, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:260, and where b is greater than or equal to a + 14.	R98775, H86395, W03494, W21603, W39528, AA013007
830109	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2938 of SEQ ID NO:261, b is an integer of 15 to 2952, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:261, and where b is greater than or equal to a + 14.	
830176	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1353 of SEQ ID NO:262, b is an integer of 15 to 1367, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:262, and where b is greater than or equal to a + 14.	T55657, T55577, R47955, R48067, R49934, R55193, R55196, R74030, R74066, R74121, R74165, N39617, N46765, W52387, W53032, AA181872, AA210720, AA235708, AA427672, AA428451, AA587689, AA593989, AA580508, AA738289, AA863320, AA985417
830241	Preferably excluded from the present invention are one or more polynucleotides comprising a	T50395, T52656, T52657, T59679, T59815, T71267, T71401, T83949.

	nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2972 of SEQ ID NO:263, b is an integer of 15 to 2986, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:263, and where b is greater than or equal to a + 14.	R08594, R08686, T82168, T85364, T85403, T85462, T99588, R19848, R23594, R26150, R30987, R34212, R34329, R45949, R45949, R71572, R76884, R77046, R78812, R79310, R79674, R79863, R81331, R81582, H06174, H06438, H09357, H09416, H78585, H78637, H93997, H95034, H96313, H98061, N22977, N31015, N32393, N33210, N40268, N41923, N55580, N62347, N63268, N69012, N77082, N78438, N78878, N79109, N99916, W00679, W03474, W05350, W06941, W06854, W15351, W19918, W25292, W25298, W31697, W32150, W32002, W39443, W56864, W72121, W77845, N90421, N91264, AA131531, AA131605, AA150778, AA150886, AA165100, AA165080, AA164538, AA164685, AA226732, AA227473, AA533788, AA558790, AA738250, AA767460, AA808772, AA863422, AA876634, AA888217, AA902465, AA917896, AA948725, AA977275, AI083708, C01143, N90337
830264	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1013 of SEQ ID NO:264, b is an integer of 15 to 1027, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:264, and where b is greater than or equal to a + 14.	
830402	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1547 of SEQ ID NO:265, b is an integer of 15 to 1561, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:265, and where b is greater than or equal to a + 14.	T48752, T48751, T93134, T93241, R34614, R34615, R77506, H27565, H27647, W33042, W33093, W33104, AA034191, AA521157, AA552029, AA878639, AI000768, AI052421
830414	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1572 of SEQ ID NO:266, b is an integer of 15 to 1586, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:266, and where b is greater than or equal to a + 14.	
830444	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 758 of SEQ ID NO:267, b is an integer of 15 to	

	772, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:267, and where b is greater than or equal to a + 14.	
830476	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2468 of SEQ ID NO:268, b is an integer of 15 to 2482, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:268, and where b is greater than or equal to a + 14.	
830624	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2480 of SEQ ID NO:269, b is an integer of 15 to 2494, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:269, and where b is greater than or equal to a + 14.	
830643	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1813 of SEQ ID NO:270, b is an integer of 15 to 1827, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:270, and where b is greater than or equal to a + 14.	T58947, T58886, H05457, H07007, H14769, H15494, H43780, W47128, W47090, AA236593, AA236594, AA278666, AA278197, AA280763, AA552030, AA569812, AA570495, AA847858
830714	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3712 of SEQ ID NO:271, b is an integer of 15 to 3726, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:271, and where b is greater than or equal to a + 14.	T66210, R54846, R54610, R80475, H11702, H89352, H89538, H89545, H89352, N92237, AA088248, AA088648, AA152243, AA152209, AA232083, AA232084, AA281189, AA288012, AA419484, AA419611, AA635556, AA658115, AA731115, AA767864, AA902794, AA922587, N84692
830826	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 642 of SEQ ID NO:272, b is an integer of 15 to 656, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:272, and where b is greater than or equal to a + 14.	
830888	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1163 of SEQ ID NO:273, b is an integer of 15 to 1177, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:273, and where b is greater than or equal to a + 14.	
830984	Preferably excluded from the present invention are	

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1339 of SEQ ID NO:274, b is an integer of 15 to 1353, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:274, and where b is greater than or equal to a + 14.	
831015	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2648 of SEQ ID NO:275, b is an integer of 15 to 2662, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:275, and where b is greater than or equal to a + 14.	
831080	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2540 of SEQ ID NO:276, b is an integer of 15 to 2554, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:276, and where b is greater than or equal to a + 14.	
831101	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1792 of SEQ ID NO:277, b is an integer of 15 to 1806, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:277, and where b is greater than or equal to a + 14.	T51286, T51410, T39928, T76990, T77156, T77499, R33812, R33813, R38383, H21687, H21714, H21898, H21919, H25832, H26197, H26795, H28008, H28649, H28869, H44418, H45258, H45325, H92329, H95779, AA043477, AA043478, AA054267, AA054080, AA257073, AA257167, AA458482, AA459283, AA459512, AA425918, AA428787, AA287938, AA288002, AA505764, AA558609, AA743768, AA805217, AA894751, AA954931, AA976613, A1056442, A1074512, F19600, C00516, C17221, C19044
831146	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2494 of SEQ ID NO:278, b is an integer of 15 to 2508, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:278, and where b is greater than or equal to a + 14.	
831215	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2398 of SEQ ID NO:279, b is an integer of 15 to 2412, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	



	NO:279, and where b is greater than or equal to a + 14.	
831231	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3558 of SEQ ID NO:280, b is an integer of 15 to 3572, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:280, and where b is greater than or equal to a + 14.	
831242	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2347 of SEQ ID NO:281, b is an integer of 15 to 2361, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:281, and where b is greater than or equal to a + 14.	R24850, R44553, R44553, N28609
831267	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1573 of SEQ ID NO:282, b is an integer of 15 to 1587, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:282, and where b is greater than or equal to a + 14.	
831272	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1959 of SEQ ID NO:283, b is an integer of 15 to 1973, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:283, and where b is greater than or equal to a + 14.	
831291	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1048 of SEQ ID NO:284, b is an integer of 15 to 1062, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:284, and where b is greater than or equal to a + 14.	
831382	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1405 of SEQ ID NO:285, b is an integer of 15 to 1419, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:285, and where b is greater than or equal to a + 14.	
831624	Preferably excluded from the present invention are	

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1944 of SEQ ID NO:286, b is an integer of 15 to 1958, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:286, and where b is greater than or equal to a + 14.	
831640	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1216 of SEQ ID NO:287, b is an integer of 15 to 1230, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:287, and where b is greater than or equal to a + 14.	
831688	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1623 of SEQ ID NO:288, b is an integer of 15 to 1637, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:288, and where b is greater than or equal to a + 14.	T50037, R16008, R28438, R35855, R70096, H12528, H21713, H27583, R92877, N31160, N64728, N95336, W04892, W24359, W39124, W56834, W61228, W76089, AA000992, AA054070, AA057867, AA128735, AA157619, AA157633, AA186509
831690	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3294 of SEQ ID NO:289, b is an integer of 15 to 3308, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:289, and where b is greater than or equal to a + 14.	
831718	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2225 of SEQ ID NO:290, b is an integer of 15 to 2239, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:290, and where b is greater than or equal to a + 14.	
831832	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1502 of SEQ ID NO:291, b is an integer of 15 to 1516, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:291, and where b is greater than or equal to a + 14.	
831907	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	T57273, R50749, H87724, AA236748, AA252480, AA252512, AA279648, AA279856, AA512986, AA593101, AA742353, AA806266, AA830807,

	2195 of SEQ ID NO:292, b is an integer of 15 to 2209, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:292, and where b is greater than or equal to a + 14.	AA838419, AA878541, A1089406
831938	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2057 of SEQ ID NO:293, b is an integer of 15 to 2071, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:293, and where b is greater than or equal to a + 14.	
831954	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1837 of SEQ ID NO:294, b is an integer of 15 to 1851, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:294, and where b is greater than or equal to a + 14.	AA425659, AA427784, AA603348, AA740730, AA746891, AA767876, AA768318, AA811192
832028	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2984 of SEQ ID NO:295, b is an integer of 15 to 2998, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:295, and where b is greater than or equal to a + 14.	
832043	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1268 of SEQ ID NO:296, b is an integer of 15 to 1282, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:296, and where b is greater than or equal to a + 14.	
832055	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 664 of SEQ ID NO:297, b is an integer of 15 to 678, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:297, and where b is greater than or equal to a + 14.	T55840, T65026, T85378, T97664, R21752, R38028, R38114, R79190, R79933, H27252, H28594, H38179, H52504, H61762, H64985, H64984, H80983, H64985, W19298, W38161, W45410, W60075, W73887, AA010631, AA035576, AA037696, AA037722, AA043216, AA043217, AA085492, AA088439, AA129575, AA129574, AA136658, AA136645, AA147228, AA148284, AA155661, AA157944, AA182640, AA190966, AA191414, AA502832, AA524526, AA581093, AA603586, AA627686, AA662517, AA903050, AA962397, AA988297, W60032, C05782, C06111,

		C06123, C06365, C16377
832124	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1668 of SEQ ID NO:298, b is an integer of 15 to 1682, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:298, and where b is greater than or equal to a + 14.	
832145	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1580 of SEQ ID NO:299, b is an integer of 15 to 1594, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:299, and where b is greater than or equal to a + 14.	
832254	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1088 of SEQ ID NO:300, b is an integer of 15 to 1102, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:300, and where b is greater than or equal to a + 14.	
832331	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1075 of SEQ ID NO:301, b is an integer of 15 to 1089, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:301, and where b is greater than or equal to a + 14.	
832360	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1270 of SEQ ID NO:302, b is an integer of 15 to 1284, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:302, and where b is greater than or equal to a + 14.	
832401	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1095 of SEQ ID NO:303, b is an integer of 15 to 1109, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:303, and where b is greater than or equal to a + 14.	R44301, R44301, R64177, R67317, H14557, H14558, H95697, H98099, N76143, N80185, N91919, W03620, N89751, AA037403, AA043199, AA115195, AA126984, AA287843, AA470665, AA713676, AA836329
832403	Preferably excluded from the present invention are one or more polynucleotides comprising a	

	nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 574 of SEQ ID NO:304, b is an integer of 15 to 588, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:304, and where b is greater than or equal to a + 14.	
832437	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2005 of SEQ ID NO:305, b is an integer of 15 to 2019, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:305, and where b is greater than or equal to a + 14.	
832492	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3303 of SEQ ID NO:306, b is an integer of 15 to 3317, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:306, and where b is greater than or equal to a + 14.	T39695, T93340, T94018, R37464, R48985, R81197, R81306, H09730, H10488, H20077, H26355, H44167, H66653, H66652, H93441, H95634, N21122, N31137, N36102, N39058, N39056, N41726, N44108, N48248, N62579, N69937, N73073, N73085, N75034, N75419, N80353, N98402, N98614, W02296, W02312, W05712, W19510, W56426, W56310, W56311, W56353, W56456, W60626, W60627, W94951, W95848, W96132, W96133, N89916, AA031971, AA121379, AA121380, AA126802, AA129078, AA129079, AA149578, AA259087, AA429410, AA429457, AA494332, AA602997, AA640565, AA731854, AA766500, AA769717, AA824556, AA824560, AA825584, AA825924, AA831365, AA857427, AA864804, AA877673, AA886291, AA888506, AA948257, AA954718, AA962473, AA962630, A1024764, C00923
832598	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1269 of SEQ ID NO:307, b is an integer of 15 to 1283, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:307, and where b is greater than or equal to a + 14.	AA179189, AA179199, AA483506, AA551887, AA631189, AA806513, AA837535
832605	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4239 of SEQ ID NO:308, b is an integer of 15 to 4253, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:308, and where b is greater than or equal to a + 14.	
834510	Preferably excluded from the present invention are	AA036881, AA593656, AA749013,

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2169 of SEQ ID NO:309, b is an integer of 15 to 2183, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:309, and where b is greater than or equal to a + 14.	AA885587, AA953834, AI089760, AI097541
835139	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3078 of SEQ ID NO:310, b is an integer of 15 to 3092, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:310, and where b is greater than or equal to a + 14.	R20799, R20912, R24679, R25179, R27752, R27753, R34881, R34983, R62621, R62672, R63644, R63645, R63707, R63754, R64165, R66336, R66337, R67478, R76113, R79475, R79947, R80040, R80239, R80347, R81703, R81704, H00296, H00334, H03690, H06286, H06338, H15772, H15773, H17464, H17570, H18473, H18581, H27396, H59355, H60010, H60011, H81738, H81739, H85151, H99488, N22740, N27287, N29648, N40024, N44259, N56603, N66901, N67813, N73296, N78755, N79053, W02360, W04650, W16638, W23994, W31574, AA026935, AA029759, AA030015, AA037453, AA037539, AA074776, AA075032, AA082470, AA125847, AA125848, AA133803, AA133804, AA171700, AA171624, AA460439, AA460440, AA229613, AA229704, AA490407, AA507836, AA555000, AA558375, AA581610, AA583156, AA614396, AA622078, AA622792, AA573324, AA575970, AA658946, AA662616, AA661494, AA742747, AA746025, AA747027, AA828231, AA911854, AA932862, AA932967, AA953903, AA969763, AA973490, AA974858, D82805, N56443, W03397, N87782, C17301, AA093368, AA093741
835142	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1282 of SEQ ID NO:311, b is an integer of 15 to 1296, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:311, and where b is greater than or equal to a + 14.	
835271	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1334 of SEQ ID NO:312, b is an integer of 15 to 1348, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:312, and where b is greater than or equal to a +	

	14.	
835369	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 399 of SEQ ID NO:313, b is an integer of 15 to 413, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:313, and where b is greater than or equal to a + 14.	
835430	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1729 of SEQ ID NO:314, b is an integer of 15 to 1743, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:314, and where b is greater than or equal to a + 14.	
835462	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2030 of SEQ ID NO:315, b is an integer of 15 to 2044, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:315, and where b is greater than or equal to a + 14.	
835539	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1736 of SEQ ID NO:316, b is an integer of 15 to 1750, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:316, and where b is greater than or equal to a + 14.	
835635	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2369 of SEQ ID NO:317, b is an integer of 15 to 2383, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:317, and where b is greater than or equal to a + 14.	R08753, R08753, R18799, H17931, H92797, N26921, N39878, AA069527, AA069528, AA192726, AA527342, AA594555, AA744123, AA806333, AA804403, AA811410, AA834380, AA911900, AA928410, AA976336, A1054047
835815	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1047 of SEQ ID NO:318, b is an integer of 15 to 1061, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:318, and where b is greater than or equal to a + 14.	N34655, N51635, AA146803, AA635825, AA661648, AA744678, AA767727, AA829571, AA878646, AA887947, AA962414
836161	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general	

	formula of a-b, where a is any integer between 1 to 2358 of SEQ ID NO:319, b is an integer of 15 to 2372, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:319, and where b is greater than or equal to a + 14.	
836213	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 424 of SEQ ID NO:320, b is an integer of 15 to 438, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:320, and where b is greater than or equal to a + 14.	
836371	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2881 of SEQ ID NO:321, b is an integer of 15 to 2895, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:321, and where b is greater than or equal to a + 14.	R51309, R51421, H92393, AA027066, AA029900, AA029988, AA121315, AA121458, AA235804, AA235805, AA528009
836618	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1161 of SEQ ID NO:322, b is an integer of 15 to 1175, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:322, and where b is greater than or equal to a + 14.	
836895	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3564 of SEQ ID NO:323, b is an integer of 15 to 3578, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:323, and where b is greater than or equal to a + 14.	T58153, H52653, H54656, H54657, H63551, H63595, H64019, H64073, H91160, H91211, W40235, W45471, W86085, W86141, AA079853, AA081692, AA082043, AA136424, AA166716, AA166806, AA232636, AA417255, AA278231, AA465183, AA482770, AA485036, AA485151, AA543054, AA580845, AA582157, AA632202, AA580595, AA580712, AA714219, AA730742, AA731716, AA749004, AA761750, AA805016, AA804371, AA810686, AA811573, AA912023, AA933881, AA953645, N84915, N84914, AA094644, AA219263
837181	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1701 of SEQ ID NO:324, b is an integer of 15 to 1715, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:324, and where b is greater than or equal to a + 14.	



837238	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1674 of SEQ ID NO:325, b is an integer of 15 to 1688, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:325, and where b is greater than or equal to a + 14.	
837337	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1618 of SEQ ID NO:326, b is an integer of 15 to 1632, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:326, and where b is greater than or equal to a + 14.	
837530	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2208 of SEQ ID NO:327, b is an integer of 15 to 2222, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:327, and where b is greater than or equal to a + 14.	
837551	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2153 of SEQ ID NO:328, b is an integer of 15 to 2167, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:328, and where b is greater than or equal to a + 14.	R32620, H14146, H14152, H14176, H23134, H23135, H39661, H40959, H43793, H89978, N95385, W74136, W79674, AA148653, AA148866, AA176600, AA176831, AA176853, AA176960, AA195247, AA195432, AA243640, AA243759, AA258378, AA458602, AA463989, AA464129, AA418426, AA514447, AA515681, F17274, AA745713, AA748828, AA768685, AA828210, AA865669, AA894376, AA906678, AA973976, AA975159, AA977112, AA989448, AA996318, A1084115, C03377, AA291674, AA292183, AA399227
837622	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2359 of SEQ ID NO:329, b is an integer of 15 to 2373, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:329, and where b is greater than or equal to a + 14.	
839908	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1355 of SEQ ID NO:330, b is an integer of 15 to 1369, where both a and b correspond to the	H00388

	positions of nucleotide residues shown in SEQ ID NO:330, and where b is greater than or equal to a + 14.	
839949	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2850 of SEQ ID NO:331, b is an integer of 15 to 2864, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:331, and where b is greater than or equal to a + 14.	
840000	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1971 of SEQ ID NO:332, b is an integer of 15 to 1985, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:332, and where b is greater than or equal to a + 14.	N31178, AA127053, AA127054, AA158523, AA458650, AA429099, AA533105, AA632061, AA804959, A1083728, AA641620, C15514, AA482401, AA482546, D20434
840095	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3073 of SEQ ID NO:333, b is an integer of 15 to 3087, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:333, and where b is greater than or equal to a + 14.	
840166	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 884 of SEQ ID NO:334, b is an integer of 15 to 898, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:334, and where b is greater than or equal to a + 14.	
840249	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 930 of SEQ ID NO:335, b is an integer of 15 to 944, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:335, and where b is greater than or equal to a + 14.	
840601	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1593 of SEQ ID NO:336, b is an integer of 15 to 1607, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:336, and where b is greater than or equal to a + 14.	
840613	Preferably excluded from the present invention are one or more polynucleotides comprising a	

	nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3142 of SEQ ID NO:337, b is an integer of 15 to 3156, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:337, and where b is greater than or equal to a + 14.	
840699	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1001 of SEQ ID NO:338, b is an integer of 15 to 1015, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:338, and where b is greater than or equal to a + 14.	
840752	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2074 of SEQ ID NO:339, b is an integer of 15 to 2088, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:339, and where b is greater than or equal to a + 14.	
840755	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3110 of SEQ ID NO:340, b is an integer of 15 to 3124, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:340, and where b is greater than or equal to a + 14.	<p>T57108, T57176, T74743, T77315, T78379, R12958, R20783, R20892, R21751, R21796, R40153, R45262, R77721, R78054, R78258, R78259, H09712, H09767, H09853, H11767, H12018, H15956, H23057, H23153, H23152, H24337, R99189, R99188, H50714, H50818, H80858, H80954, H88936, H89165, N20086, N23817, N26014, N26287, N26603, N28761, N28817, N30771, N34519, N36179, N36646, N39466, N44167, N48755, N66360, N94369, W73964, W79270, W84318, W96356, W96355, AA011090, AA011089, AA019540, AA018804, AA022750, AA022852, AA025733, AA025734, AA039842, AA045071, AA045453, AA055314, AA055315, AA083189, AA083190, AA084374, AA088698, AA088835, AA101934, AA101933, AA127099, AA127242, AA125914, AA126525, AA129220, AA151769, AA149728, AA149794, AA150111, AA156586, AA191756, AA221003, AA461267, AA226380, AA226492, AA278931, AA470412, AA505893, AA513664, AA515582, AA564504, AA594361, AA631351, AA580020, AA812186, AA830236, AA831237, AA858302, AA946744, W84345, C04338, C05340, C14190, AA214461, AA218996,</p>

		AA403162, AA403230, AA421391, AA488608, AA599592, AA633970, AA703929, AA723222, AA775157, AA778160, AA779754, AA781965, AA853681, AA853682, AA905632, AA906459, AA907720, A1031789, A1095882, T11252, T11253, D20806, Z39978, Z44490, T19212, T19356, T19426, F03570, F03732, F06913, F07503, F00825, F12904, F11081, F10946, F13350
840844	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 231 of SEQ ID NO:341, b is an integer of 15 to 245, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:341, and where b is greater than or equal to a + 14.	W79632
841066	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 5654 of SEQ ID NO:342, b is an integer of 15 to 5668, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:342, and where b is greater than or equal to a + 14.	
841306	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 800 of SEQ ID NO:343, b is an integer of 15 to 814, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:343, and where b is greater than or equal to a + 14.	
841913	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 887 of SEQ ID NO:344, b is an integer of 15 to 901, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:344, and where b is greater than or equal to a + 14.	
842025	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2574 of SEQ ID NO:345, b is an integer of 15 to 2588, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:345, and where b is greater than or equal to a + 14.	
842178	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	

	3756 of SEQ ID NO:346, b is an integer of 15 to 3770, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:346, and where b is greater than or equal to a + 14.	
842438	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2344 of SEQ ID NO:347, b is an integer of 15 to 2358, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:347, and where b is greater than or equal to a + 14.	T95189, R21912, R22561, R77669, R77668, H02572, H02656, H02698, N36870, N46443, N48944, N50609, N67667, N93157, N94539, W04700, W19929, W20226, W30813, W31802, W32098, W38692, W38737, W38971, W42987, W44880, W45246, W46417, W46443, W55884, W55885, W60493, W60779, N89986, N90587, AA031818, AA031819, AA043240, AA043569, AA057282, AA057064, AA058727, AA058543, AA069989, AA112313, AA113069, AA135730, AA135774
843289	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2030 of SEQ ID NO:348, b is an integer of 15 to 2044, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:348, and where b is greater than or equal to a + 14.	
843447	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 779 of SEQ ID NO:349, b is an integer of 15 to 793, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:349, and where b is greater than or equal to a + 14.	
843743	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1044 of SEQ ID NO:350, b is an integer of 15 to 1058, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:350, and where b is greater than or equal to a + 14.	T59832, T63706, T64557, T65980, T82436, T92853, R09362, R64003, R73660, R77168, R78819, R80999, H52262, H52359, H61317, H94744, H94791, N93055, N99151, W24688, AA126780, AA128359, AA128522, AA160539, AA160634, AA173272, AA223663, AA223749
843878	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1334 of SEQ ID NO:351, b is an integer of 15 to 1348, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:351, and where b is greater than or equal to a + 14.	
843964	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to	

	3156 of SEQ ID NO:352, b is an integer of 15 to 3170, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:352, and where b is greater than or equal to a + 14.	
844071	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2999 of SEQ ID NO:353, b is an integer of 15 to 3013, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:353, and where b is greater than or equal to a + 14.	
844444	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1815 of SEQ ID NO:354, b is an integer of 15 to 1829, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:354, and where b is greater than or equal to a + 14.	
844561	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1628 of SEQ ID NO:355, b is an integer of 15 to 1642, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:355, and where b is greater than or equal to a + 14.	
844953	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2006 of SEQ ID NO:356, b is an integer of 15 to 2020, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:356, and where b is greater than or equal to a + 14.	N51486, N53629, N59811, N72758, AA148559, AA165330, AA235159, AA489244, AA504283, AA689472, AA689529, AA714017, AA731441, C01997, N88816, A1025597
844990	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1203 of SEQ ID NO:357, b is an integer of 15 to 1217, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:357, and where b is greater than or equal to a + 14.	
845379	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1949 of SEQ ID NO:358, b is an integer of 15 to 1963, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID	

	NO:358, and where b is greater than or equal to a + 14.	
845829	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1373 of SEQ ID NO:359, b is an integer of 15 to 1387, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:359, and where b is greater than or equal to a + 14.	T77613, H29246, H63829, AA164605

*Polynucleotide and Polypeptide Variants*

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X or the complementary strand thereto, and/or the cDNA sequence contained in a cDNA clone contained in the deposit.

5       The present invention also encompasses variants of the lung and lung cancer polypeptide sequence disclosed in SEQ ID NO:Y, a polypeptide sequence encoded by the polynucleotide sequence in SEQ ID NO:X, and/or a polypeptide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

10       "Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

15       The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence of the related cDNA contained in a deposited library or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA in the related cDNA contained in a deposited library, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecules which comprise or alternatively consist of, a polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, 25       under low stringency conditions, to the nucleotide coding sequence in SEQ ID NO:X, the nucleotide coding sequence of the related cDNA clone contained in a deposited library, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a 30       nucleotide sequence encoding the polypeptide encoded by the cDNA in the related cDNA clone contained in a deposited library, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which



hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively  
5 consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,  
99% or 100% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, a  
polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a polypeptide  
sequence encoded by the cDNA in the related cDNA clone contained in a deposited library,  
and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described  
10 herein). Polynucleotides which hybridize to the complement of the nucleic acid molecules  
encoding these polypeptides under stringent hybridization conditions, or alternatively, under  
lower stringency conditions, are also encompassed by the invention, as are polypeptides  
encoded by these polynucleotides.

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical"  
15 to a reference nucleotide sequence of the present invention, it is intended that the nucleotide  
sequence of the nucleic acid is identical to the reference sequence except that the nucleotide  
sequence may include up to five point mutations per each 100 nucleotides of the reference  
nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid  
having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to  
20 5% of the nucleotides in the reference sequence may be deleted or substituted with another  
nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference  
sequence may be inserted into the reference sequence. The query sequence may be, for  
example, an entire sequence referred to in Table 1, an ORF (open reading frame), or any  
fragment specified as described herein.

25 As a practical matter, whether any particular nucleic acid molecule or polypeptide is  
at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of  
the present invention can be determined conventionally using known computer programs. A  
preferred method for determining the best overall match between a query sequence (a  
sequence of the present invention) and a subject sequence, also referred to as a global  
30 sequence alignment, can be determined using the FASTDB computer program based on the  
algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). In a sequence alignment  
the query and subject sequences are both DNA sequences. An RNA sequence can be

compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size  
5 Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the  
10 subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment.  
15 This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of  
20 manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and  
25 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which  
30 are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for.

No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence in SEQ ID NO:Y or a fragment thereof, the amino acid sequence encoded by the nucleotide sequence in SEQ ID NO:X or a fragment thereof, or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library, or a fragment thereof, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237- 245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences

truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is  
5 matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the  
10 query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the  
15 subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent  
20 identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject  
25 sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce  
30 silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which less than 50, less

than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host  
5 such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention.  
10 Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, as discussed herein, one or more amino acids can be deleted  
15 from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. The authors of Ron et al., *J. Biol. Chem.* 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the  
20 carboxy terminus of this protein. (Dobeli et al., *J. Biotechnology* 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (*J. Biol. Chem.* 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that  
25 averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in  
30 activity from wild-type.

Furthermore, as discussed herein, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more

biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show a functional activity (e.g., biological activity) of the polypeptide of the invention of which they are a variant. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein or fragments thereof, (e.g., including but not limited to fragments encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion), irrespective of whether they encode a polypeptide having functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having functional activity include, inter alia, (1) isolating a gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the gene, as described in Verma et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having a functional activity of a polypeptide of the invention.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for example, the nucleic acid sequence of the cDNA in the related cDNA clone contained in a

deposited library, the nucleic acid sequence referred to in Table 1 (SEQ ID NO:X), or fragments thereof, will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, *Science* 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side

chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X, and/or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library which contains, in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or



1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature form and/or other fragments described herein), an amino acid sequence encoded by SEQ ID NO:X or fragments thereof, and/or the amino acid sequence encoded by the cDNA  
5 in the related cDNA clone contained in a deposited library or fragments thereof, is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

#### *Polynucleotide and Polypeptide Fragments*

The present invention is also directed to polynucleotide fragments of the lung and  
10 lung cancer polynucleotides (nucleic acids) of the invention. In the present invention, a "polynucleotide fragment" refers, for example, to a polynucleotide having a nucleic acid sequence which: is a portion of the cDNA contained in a deposited cDNA clone; or is a portion of a polynucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited cDNA clone; or is a portion of the polynucleotide sequence in SEQ  
15 ID NO:X or the complementary strand thereto; or is a polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; or is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more  
20 preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, at least about 100 nt, at least about 125 nt or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from, for example, the sequence contained in the cDNA in a related cDNA clone contained in a deposited library, the nucleotide sequence shown in SEQ ID NO:X or the complementary stand thereto. In this  
25 context "about" includes the particularly recited value or a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 150, 175, 200, 250, 500, 600, 1000, or 2000 nucleotides in length) are also encompassed by the invention.

30 Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-

400, 401-450, 451-500, 501-550, 551-600, 651-700, 701- 750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, and 3551 to the end of SEQ ID NO:X, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g., biological activity) of the polypeptide encoded by the polynucleotide of which the sequence is a portion. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701- 750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, and 3551 to the end of the cDNA nucleotide sequence contained in the deposited cDNA clone, or the complementary strand thereto. In this context "about" includes the particularly recited range, or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a

functional activity (e.g., biological activity) of the polypeptide encoded by the cDNA nucleotide sequence contained in the deposited cDNA clone. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these fragments under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y, a portion of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO:X, and/or encoded by the cDNA contained in the related cDNA clone contained in a deposited library. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, an amino acid sequence from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, 781-800, 801-820, 821-840, 841-860, 861-880, 881-900, 901-920, 921-940, 941-960, 961-980, 981-1000, 1001-1020, 1021-1040, 1041-1060, 1061-1080, 1081-1100, 1101-1120, 1121-1140, 1141-1160, 1161-1180, and 1181 to the end of SEQ ID NO:Y. Moreover, polypeptide fragments of the invention may be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, or ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either terminus or at both termini. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained

when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a  
5 mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, polypeptide fragments of the invention include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted  
10 protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above  
15 amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide  
20 sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in the related cDNA clone contained in a deposited library). In particular, N-terminal deletions may be described by the general formula  $m-q$ , where  $q$  is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and  $m$  is defined as any integer ranging from 2  
25 to  $q-6$ . Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example the ability of the shortened mutein  
30 to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular

polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

5 Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained  
10 in deposited cDNA clone referenced in Table 1). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

15 In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to, the preferred polypeptide disclosed as SEQ ID  
20 NO:Y), and/or the cDNA in the related cDNA clone contained in a deposited library, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Any polypeptide sequence contained in the polypeptide of SEQ ID NO:Y, encoded by the polynucleotide sequences set forth as SEQ ID NO:X, or encoded by the cDNA in the  
25 related cDNA clone contained in a deposited library may be analyzed to determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of SEQ ID NO:X, or the cDNA in a deposited cDNA clone may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; <http://www.dnastar.com/>).

30 Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions,

Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides of the invention in this regard are those that encode polypeptides comprising regions that  
5 combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing  
10 four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the  
15 process of initiation of an immune response.

Preferred polypeptide fragments of the invention are fragments comprising, or alternatively consisting of, an amino acid sequence that displays a functional activity of the polypeptide sequence of which the amino acid sequence is a fragment.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide  
20 capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the  
25 invention, and ability to bind to a receptor or ligand for a polypeptide.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

30 In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of

SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Table 4.

Sequence/ Contig ID	Epitope
507002	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 444 as residues: Gln-15 to Gln-34, Ser-40 to Gly-52, Gly-80 to Met-85, Ser-95 to Lys-100, Gln-107 to Lys-113, Asp-131 to Glu-141, Gln-206 to Pro-228, Ser-235 to Met-240, Val-242 to Lys-249, Ser-255 to Gly-262, Cys-309 to Thr-323.
508935	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 445 as residues: Arg-3 to Thr-8, Glu-32 to Glu-37, Ser-46 to Ala-54, Ala-110 to Gly-116, Gly-234 to Glu-239, Lys-276 to His-282, Thr-342 to Pro-348, Lys-410 to Gln-415, Pro-428 to Lys-437, Arg-446 to Thr-452, Asp-527 to Leu-533, Pro-548 to Glu-556, Glu-563 to Tyr-568, Gly-579 to Val-586.
518959	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 446 as residues: Ser-51 to Gly-58, Thr-70 to Gly-76, Pro-105 to Trp-110.
539756	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 447 as residues: Pro-11 to Pro-17.
540125	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 448 as residues: Pro-9 to His-14, Asn-21 to His-27, Val-276 to Glu-285, Thr-308 to Asp-335.
540275	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 449 as residues: Ser-14 to Gly-20, Tyr-45 to Ser-55, Gly-91 to Ser-99, Thr-123 to Ser-128, Thr-134 to Glu-142, Arg-189 to Lys-202, Glu-225 to Gly-230, Ser-237 to Ser-245, Lys-432 to Trp-439, Ile-471 to Asp-476, Glu-575 to Phe-581, Thr-602 to Ala-608.
540331	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 450 as residues: Ser-22 to Cys-34, Gln-47 to Ser-54, Glu-61 to Gly-67, Pro-69 to Trp-78.
540955	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 451 as residues: Met-43 to Ser-49, Leu-107 to Gly-114, Gly-130 to Ser-141.
541251	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 452 as residues: Gly-60 to Leu-66, Gln-139 to Gly-146, Leu-165 to Arg-177, Arg-192 to Trp-199, Gln-248 to Thr-253, Leu-257 to Asn-270, Leu-344 to Pro-351, Ala-398 to Gly-409, Glu-466 to Arg-486.
541978	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 453 as residues: Gln-7 to Arg-12, Ser-64 to Lys-72, Ala-108 to Glu-113, Arg-127 to Gln-141.
547680	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 454 as residues: Asp-12 to Tyr-17, Asn-56 to Gly-65, Ala-70 to Thr-80, Ile-85 to Phe-94, Thr-96 to Tyr-101, Ala-114 to Ser-120, Glu-126 to Arg-131, Thr-143 to Gly-148, Asp-192 to Tyr-198, Ile-212 to Tyr-219.
547705	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 455 as residues: Asn-1 to Val-8, Gln-26 to Ser-31, Asp-50 to Tyr-56, Arg-70 to Trp-75, Val-87 to Leu-93, Glu-106 to Asp-112, Pro-126 to Asp-135, Ser-203 to His-208, Gln-222 to Gly-236, Ser-244 to Trp-254.
549819	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 457 as residues: His-1 to His-11.
549820	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 458 as residues: Ser-27 to Glu-43, Leu-75 to Phe-83.
551426	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 460 as residues: Glu-1 to Ala-8, Gly-12 to Lys-37, Ser-91 to Arg-100.
552182	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 461 as residues: Glu-24 to Cys-29, Ser-58 to Val-63.
552540	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 462 as residues: Gly-2 to Pro-8, Pro-57 to Arg-65.
553367	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 463 as residues: Arg-2 to Arg-13, Ala-32 to Gly-44, Ala-52 to Gly-59, His-85 to Lys-97,



	Ala-160 to Ser-166, Ser-188 to Ile-193, Asp-209 to Phe-232.
554326	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 464 as residues: Arg-1 to Pro-15, Ser-146 to Arg-155, Leu-168 to Asp-174, Lys-181 to Thr-186.
554657	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 465 as residues: Asp-45 to Pro-56, Thr-86 to Ser-91, Pro-127 to Arg-133, Asp-201 to Gln-215.
556156	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 466 as residues: Asp-44 to Val-52, His-71 to Ile-77.
557747	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 467 as residues: Tyr-18 to Ile-39, Asp-42 to Ala-48, Pro-71 to Glu-76, Ser-109 to Glu-119, Glu-133 to Thr-142.
558599	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 468 as residues: Lys-5 to Ala-11, Pro-13 to Gly-22, Pro-68 to Gln-73, Gly-99 to Asn-108, Lys-137 to His-149.
573366	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 470 as residues: Ser-2 to Ala-13.
573986	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 471 as residues: Pro-10 to Gly-18, Glu-25 to Thr-37.
575435	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 472 as residues: Gly-65 to Tyr-75, Asp-86 to Glu-91, Phe-120 to Gly-125, Leu-135 to Asn-148, Trp-256 to Leu-261, Ser-309 to Ser-314, Glu-346 to Thr-354, Met-361 to Asp-366.
584435	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 474 as residues: Gly-5 to Met-13, Tyr-137 to Thr-143, Gly-161 to Gly-168, Gln-221 to Ser-226.
585658	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 476 as residues: Ser-16 to Leu-25, Ala-103 to Asp-108, Ser-128 to Ser-139.
585693	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 477 as residues: Gln-28 to Lys-34, Leu-148 to Leu-154, Val-205 to Thr-210.
585701	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 478 as residues: Ala-17 to Gln-24, Lys-70 to Glu-79, Leu-124 to Tyr-145, Val-161 to Ala-166, Gln-203 to Gly-212, Asp-232 to Gly-248, Thr-299 to Lys-307.
586019	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 479 as residues: Val-104 to Ala-123, Gly-173 to Glu-180, Arg-197 to Phe-204.
587225	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 480 as residues: Glu-58 to Pro-65, Gln-74 to Cys-81, Ile-111 to Gln-119, Glu-147 to Trp-152, Pro-162 to Gln-167, Phe-208 to Ala-215, Asp-222 to Thr-228, Phe-230 to Gly-235, Tyr-250 to Pro-257, Lys-272 to Leu-278.
587445	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 481 as residues: Pro-50 to Arg-55, Leu-68 to Arg-73.
587596	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 483 as residues: Gln-29 to Pro-35, Asn-51 to Glu-57.
588548	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 484 as residues: Ile-88 to Phe-97, Lys-132 to Trp-137, Gly-169 to Asp-184.
588881	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 485 as residues: Leu-9 to Thr-17, Ser-56 to Trp-62, Asp-93 to Asp-101, Thr-249 to Thr-255.
588933	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 486 as residues: His-1 to Ser-6, Trp-29 to Pro-35, Asp-37 to Gly-43, Thr-45 to Leu-61, Lys-72 to Thr-77, Glu-83 to Tyr-90, His-129 to Gln-135.
592136	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 487 as residues: His-1 to Thr-8, Arg-22 to Thr-28, Met-46 to Asn-51.
613777	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 488 as residues: Ala-16 to Glu-27, Lys-34 to Ser-48, Cys-54 to Thr-61, Cys-120 to Trp-128.
614669	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 489 as residues: Glu-1 to Arg-10, Ser-17 to Gly-23, Asp-49 to Lys-54, Glu-71 to Val-78.

	Asp-99 to Gly-104, Asp-156 to Arg-161, Gln-211 to Cys-220, Ser-234 to Tyr-239, Ser-254 to Arg-264.
619502	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 490 as residues: Glu-4 to Glu-25, Leu-39 to Val-45, Leu-49 to Glu-62, Gly-73 to Lys-83.
619525	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 491 as residues: Glu-24 to Gly-47, Gln-196 to Ala-202, Ala-234 to Arg-239.
623660	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 492 as residues: Val-12 to Asn-22, Pro-95 to Gly-100, Leu-118 to Ser-132.
625480	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 493 as residues: Gly-23 to Ser-33.
647688	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 494 as residues: Gly-1 to Leu-7.
650865	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 495 as residues: Asp-1 to Ala-7.
651676	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 496 as residues: Ile-55 to Asp-60, Glu-82 to Lys-94, Glu-115 to Asp-128.
651751	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 497 as residues: Ala-1 to Thr-8, Arg-15 to Ser-22, Arg-122 to Gly-138, Gln-145 to Lys-156.
651840	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 499 as residues: Pro-1 to Glu-6, Pro-16 to Ser-21, Pro-23 to Tyr-31, Asp-48 to Val-60, Phe-68 to Lys-76, Tyr-79 to Asn-93, Lys-105 to Ser-114, Gly-125 to Gly-130, Asp-160 to Leu-175, Asn-179 to Pro-188, Val-196 to Lys-203, Pro-209 to Arg-217, Ser-243 to Leu-252, Arg-274 to Ile-282, Ser-368 to Phe-373, Gln-383 to Gly-388, Asn-403 to Gly-408, Glu-429 to Ile-438.
652557	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 501 as residues: Gly-55 to Leu-62, Ala-80 to Ile-87, Arg-110 to Arg-118.
653011	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 502 as residues: Ser-47 to Cys-57.
656930	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 504 as residues: His-13 to Asn-18, Met-40 to Arg-45, Asp-73 to Lys-80, Glu-85 to Glu-90, Gln-97 to Cys-107, Gln-119 to Ala-124, Thr-188 to Trp-194, Asp-241 to Cys-251.
659023	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 505 as residues: Ile-1 to Ala-19.
660696	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 507 as residues: Val-1 to Gly-9, Pro-17 to Gly-24, Gly-39 to Gly-45, Lys-65 to Asp-70.
666881	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 508 as residues: Pro-2 to Gly-7.
681507	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 511 as residues: Ala-6 to Cys-18, Pro-71 to Gly-87, Ile-95 to Val-101, Ser-104 to Lys-112, Glu-117 to Ala-125, Gly-127 to Glu-142, Pro-150 to Ala-164, Leu-168 to Glu-187.
683116	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 513 as residues: Pro-1 to Gly-15.
686494	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 514 as residues: Pro-10 to Glu-19, Asn-46 to Arg-52.
688221	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 516 as residues: Arg-4 to Gly-17, Pro-39 to Lys-46, His-96 to Arg-102, Ala-214 to Ile-222, Glu-247 to Lys-255.
705227	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 519 as residues: Lys-6 to Trp-11, Lys-32 to Glu-37, Lys-48 to Thr-54.
705958	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 520 as residues: Pro-1 to Glu-14, Ala-25 to Ala-32.
705965	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 521 as residues: Gly-14 to Gly-22, Gln-35 to Arg-60, Thr-70 to Lys-80, Arg-87 to Ala-99.
707380	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 524 as residues: Leu-1 to Ala-7.
707779	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 525 as

	residues: Ser-9 to Pro-26, Ala-57 to Asp-66, Thr-76 to Gly-81, Pro-93 to Glu-101, Phe-111 to Phe-124, Glu-145 to Trp-150, Pro-170 to Ala-176.
709441	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 526 as residues: Glu-8 to Ala-18, Pro-60 to Glu-66, Val-71 to Arg-76.
710443	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 527 as residues: Thr-28 to Ser-40, Pro-100 to Leu-105, Arg-123 to Ser-129, Lys-153 to Asn-162, Arg-171 to Lys-182, Pro-228 to Pro-245, Ser-249 to Ser-257, Ser-279 to Pro-288, Val-297 to Glu-322, Val-335 to Asn-340.
710616	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 529 as residues: His-1 to Gly-12, Gln-76 to Tyr-83, Ile-93 to Ser-98, Pro-116 to Ser-123, Gln-159 to Gln-164.
710662	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 530 as residues: Asn-12 to Val-17, Gly-24 to Val-29, Lys-56 to Val-67, Pro-69 to Thr-74, Arg-78 to Gly-87.
710917	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 531 as residues: Glu-1 to Gly-10, Gly-78 to Thr-87, Asn-124 to Arg-136, Ser-223 to Asp-244, Lys-247 to Thr-252, Asp-265 to Gly-274, Glu-279 to Ile-312, Thr-334 to Glu-340, Gln-345 to Gln-350, Arg-356 to Glu-368, Asn-375 to Arg-381, Glu-398 to Leu-406, Ser-435 to His-441, Ala-453 to Arg-458, Glu-492 to Ser-497, Leu-519 to Asp-525, Ser-543 to Glu-549, Pro-563 to Gly-569, Ser-587 to Asp-592, Glu-617 to Ser-622, Arg-659 to Glu-664, Leu-677 to His-683, Asn-685 to Lys-698.
711866	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 532 as residues: Arg-27 to Arg-33, Ser-35 to Gln-40.
714903	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 533 as residues: Arg-2 to Ile-7.
718139	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 534 as residues: Phe-1 to Glu-7, Pro-22 to Tyr-27, Ala-108 to Lys-114, Thr-134 to Phe-139, Ala-170 to Ala-183, Ser-216 to Asp-231, Cys-235 to Ser-244.
719142	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 535 as residues: Ser-31 to Gly-37, Pro-39 to Pro-44, Ile-66 to Trp-71, Ser-117 to Leu-123.
719914	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 537 as residues: Pro-77 to Cys-85, Asp-195 to Lys-214, Pro-231 to Thr-238.
720134	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 538 as residues: Arg-13 to Ser-19.
720583	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 540 as residues: Met-18 to Ser-26, Ile-35 to Lys-47, Glu-52 to Gln-57, Arg-71 to Asp-79.
720904	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 541 as residues: Ala-72 to Arg-80, Trp-88 to Tyr-94, Tyr-112 to Met-118, Asp-131 to Val-138, Lys-168 to Lys-173.
721194	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 542 as residues: Asp-39 to Lys-44, Ala-115 to Thr-122.
721271	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 543 as residues: Lys-24 to Gly-32, Pro-53 to Leu-59.
723886	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 544 as residues: Thr-16 to Ser-22, Pro-44 to Ser-49, Ser-53 to Phe-58.
723968	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 545 as residues: Asp-33 to Asp-65, Trp-85 to Tyr-90, Asp-101 to Gly-109.
726034	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 548 as residues: Gln-6 to Ala-11.
726602	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 549 as residues: Pro-5 to Ala-11, Pro-24 to Leu-29, Glu-45 to Ser-51.
726965	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 550 as residues: Glu-5 to Leu-17, Leu-37 to Arg-44, Gly-50 to Gly-57, Val-72 to Arg-80, Asn-94 to Lys-99, Pro-107 to Ala-113.
727809	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 551 as residues: Gly-8 to Trp-16, Asn-22 to Phe-28, Phe-68 to Arg-75, Ser-93 to Ser-101.

	Glu-114 to Ile-126, Pro-134 to Phe-143, Gly-165 to Gly-176, Lys-191 to Glu-201, Thr-218 to Lys-227, Tyr-289 to Phe-299.
731703	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 552 as residues: Pro-8 to Phe-15, His-28 to Pro-34, Gln-50 to Tyr-64, Asp-69 to Tyr-74, Lys-79 to Pro-84, Ala-95 to Thr-105.
732840	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 553 as residues: Thr-2 to Ser-10, Pro-12 to Thr-22, Val-90 to Pro-98, Ile-175 to Val-181.
733749	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 555 as residues: Ser-70 to Thr-76, Ala-94 to Thr-101, Thr-105 to Lys-115, Lys-120 to Gln-138, Lys-143 to Gly-150.
734637	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 557 as residues: Ala-35 to Ile-40, Glu-60 to Asp-65, Pro-67 to Glu-85, Ser-97 to Tyr-104.
734638	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 558 as residues: Ala-35 to Ile-40, Glu-60 to Asp-65, Pro-67 to Glu-85, Ser-97 to Tyr-104, Ile-124 to Pro-132, Thr-164 to Ser-169, Phe-301 to Asp-306, Met-354 to Asn-360, Thr-368 to Asn-377, Ser-382 to Gly-396, Asp-413 to Thr-425.
738846	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 560 as residues: Leu-35 to Arg-41.
740584	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 561 as residues: Lys-1 to Lys-16.
741213	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 562 as residues: Glu-1 to Gln-34, Lys-103 to Ile-116.
741229	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 563 as residues: Ser-2 to Gln-10, Gly-18 to Pro-24, Lys-52 to Lys-58, Ala-62 to Lys-67, Ser-74 to Arg-80, Gln-95 to Pro-104, Gly-109 to Ser-116, Ile-142 to Arg-150, Pro-164 to Ala-169, Thr-207 to Asp-215, His-235 to Asp-241, Arg-273 to Gly-278, Gln-295 to Glu-301, Ser-336 to Arg-345.
741299	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 564 as residues: Arg-1 to Lys-8, Gly-145 to Gly-155, Gly-205 to His-210, Ile-313 to His-318.
744680	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 566 as residues: Ile-11 to Gly-19, Ala-26 to Cys-39.
744705	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 567 as residues: Pro-13 to Glu-21, Ala-23 to Thr-30, Lys-78 to Ser-85, Arg-131 to Thr-139.
745337	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 568 as residues: His-126 to Leu-131, Cys-181 to Pro-186, Ala-220 to Ser-226, Leu-574 to Asp-581.
750595	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 571 as residues: Met-72 to Thr-77, Ala-87 to Lys-95.
750633	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 572 as residues: Glu-113 to Phe-132.
750766	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 573 as residues: Arg-30 to Ala-40, Lys-62 to Phe-67, Ile-84 to Asn-89, Arg-91 to Lys-100, Ile-115 to Glu-120, Gly-135 to Leu-144, Pro-146 to Ala-159, Ala-214 to Glu-219, Arg-255 to Ile-261, Pro-275 to Lys-283.
754538	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 575 as residues: Ser-6 to Glu-13, Glu-21 to Asp-31, Arg-54 to Trp-70, Leu-115 to Glu-120.
754820	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 576 as residues: Asp-5 to Lys-11, Met-75 to Lys-87, Lys-96 to Gln-102.
756565	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 577 as residues: Lys-13 to Asn-25, Glu-36 to Ser-47.
756793	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 578 as residues: Arg-26 to Lys-40.
757431	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 579 as residues: Glu-64 to Pro-71, Leu-97 to Lys-104, Ser-147 to Glu-152.
757478	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 580 as

	residues: Asp-1 to Trp-10, Ala-19 to Ser-25, Thr-31 to Ser-42, Cys-128 to Gly-135, Gly-137 to Thr-143, Pro-179 to Lys-192.
760876	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 582 as residues: Ser-35 to Pro-48, Pro-56 to Trp-65, Ser-67 to Lys-76.
761528	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 583 as residues: Pro-4 to Gly-10, Thr-38 to Lys-43, Leu-54 to Gly-59, Glu-107 to Glu-116, Pro-194 to Lys-199, Leu-207 to Asn-212, Arg-227 to Ala-239, Lys-285 to Lys-294, Glu-300 to Phe-306, Gln-315 to Tyr-327, Ala-353 to Gly-360.
764913	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 586 as residues: Glu-83 to Ser-89, Ile-127 to Lys-132, Ser-134 to Asn-140, Tyr-176 to Asn-197, Gly-217 to Ser-233.
764941	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 587 as residues: Asn-7 to Ser-12, Asn-35 to Phe-43.
765903	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 588 as residues: Leu-65 to Thr-74.
766122	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 589 as residues: Ser-1 to Gly-10, Arg-30 to Asp-36, Asp-59 to Pro-64.
766719	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 590 as residues: Gln-12 to Cys-20, His-43 to Cys-49, Leu-51 to His-64, Pro-82 to Val-88.
767941	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 592 as residues: Tyr-12 to Glu-20.
768035	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 593 as residues: Phe-30 to Pro-40.
769888	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 594 as residues: His-53 to His-59, Asn-72 to Ile-81, Glu-153 to Lys-178, Pro-186 to Ser-195.
771671	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 595 as residues: Arg-32 to Ala-37.
772876	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 596 as residues: Arg-80 to Thr-91.
773398	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 598 as residues: Pro-12 to Arg-19, Lys-29 to Val-41.
773927	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 600 as residues: Gly-84 to Leu-91, Glu-122 to Pro-136, Phe-176 to Ser-197, Lys-207 to Lys-212, Pro-222 to Glu-233, Ser-246 to Tyr-257.
774100	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 601 as residues: Gly-1 to Pro-9, Arg-26 to Asp-31, Asp-33 to Val-58, Ser-60 to Gly-65, Pro-78 to Arg-90, Ser-132 to Ser-137.
774101	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 602 as residues: Thr-73 to Glu-78.
774341	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 604 as residues: Asp-82 to Glu-92, Lys-126 to Thr-131, Tyr-140 to Leu-151, Tyr-206 to Ser-211.
774371	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 605 as residues: Pro-29 to Arg-36.
777534	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 606 as residues: Arg-11 to Arg-18, Asn-93 to Lys-98, Glu-108 to Asn-116, Pro-124 to Lys-134, Ile-303 to Glu-308, Arg-328 to Lys-334, Arg-355 to Lys-363, Arg-387 to Lys-393.
777623	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 607 as residues: Glu-14 to Thr-24.
779194	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 608 as residues: Lys-29 to Thr-42.
779387	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 609 as residues: Pro-1 to His-6.
779818	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 611 as

	residues: Pro-38 to Glu-44, Glu-67 to Cys-72, Ala-81 to Leu-86, Pro-100 to Asn-111, Asp-120 to Gly-127, Arg-150 to Cys-162, Gln-184 to Gln-191, Tyr-211 to Cys-221, Asp-242 to His-250, Cys-269 to Ser-280, Glu-292 to Trp-299.
780634	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 613 as residues: Asp-1 to Lys-6, Cys-19 to Gly-27, Glu-36 to Gln-42.
780638	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 614 as residues: Asn-3 to Val-12, Pro-27 to Leu-35, Ile-70 to Gly-79, Tyr-135 to Tyr-140, Cys-142 to Tyr-148, Ser-171 to Leu-177, Ser-199 to Ser-207.
780773	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 615 as residues: Gly-16 to Ser-32, Gly-47 to Ala-54.
780778	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 616 as residues: Tyr-12 to Thr-17.
780873	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 617 as residues: Leu-4 to Trp-12, Tyr-46 to Arg-53, Asn-108 to Asp-114.
782113	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 618 as residues: Ala-2 to Cys-7.
782153	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 619 as residues: Ser-10 to His-16, Pro-26 to Asn-31, Val-74 to Asn-88, Asp-158 to Glu-165, Ile-205 to Arg-213.
782376	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 620 as residues: Thr-1 to Ser-15.
782420	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 621 as residues: Asp-1 to Gly-8.
782672	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 622 as residues: Cys-116 to Glu-126.
783148	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 623 as residues: Asp-43 to His-52.
783510	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 624 as residues: Pro-16 to Glu-23, Gly-71 to Leu-76, Asn-83 to Asp-93, Lys-121 to Arg-132, Val-137 to Trp-142, Glu-245 to Val-252, Pro-377 to Ser-385.
783734	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 625 as residues: Leu-1 to Gln-75, Glu-79 to Ile-84, Gln-116 to Gln-123.
784201	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 626 as residues: Arg-7 to Val-14, Glu-48 to Gly-58, Ser-74 to Gln-83, Asp-101 to Asp-107, Ile-113 to Asn-118.
784381	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 627 as residues: Thr-122 to Ala-130.
784387	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 628 as residues: Glu-42 to Ser-51, Asp-74 to Pro-86, Thr-104 to Gly-110, Pro-131 to Gly-138.
784639	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 629 as residues: Arg-1 to Lys-6, Asn-31 to Lys-39, Ala-66 to Gln-72, Ser-112 to Asn-118, Ile-128 to Ala-136, Cys-144 to Asn-149, Ala-174 to Glu-180, Ile-191 to Ser-202.
784641	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 630 as residues: Asn-18 to Leu-24, Asp-42 to Gly-50, Ala-84 to Gly-94, Gly-100 to Asn-159.
785142	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 632 as residues: Val-37 to Ala-42, Lys-82 to Ala-94, Asp-110 to Gly-118, Pro-132 to Lys-141, Ser-150 to Glu-161, Pro-199 to Asp-221, Leu-223 to Ser-237, Gln-255 to Gln-269, Phe-275 to Phe-298, Gln-323 to Asp-335, Pro-343 to Ala-359, Pro-375 to Gln-384, Thr-386 to Pro-392, Pro-529 to Ile-541, Leu-552 to Val-560, Arg-578 to Ser-584, Pro-602 to Phe-611, Lys-619 to Arg-629, Glu-668 to Phe-674.
786283	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 635 as residues: Glu-16 to Gln-23.
786511	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 637 as residues: Ser-13 to Ser-24.

787330	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 638 as residues: Ala-1 to Ala-9, Pro-13 to Val-20, Asn-27 to Thr-36, Pro-44 to Asn-56, Glu-71 to Arg-76, Glu-81 to Gln-96, Pro-104 to Leu-111, Leu-115 to Gln-120, Asp-139 to Ile-149.
787377	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 639 as residues: Ala-15 to His-24, Asp-32 to Pro-42, Val-53 to Gln-58, Pro-61 to Ile-77.
787662	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 640 as residues: Pro-68 to Leu-74.
789466	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 643 as residues: Lys-1 to Asp-9, Asn-62 to Met-69, Glu-71 to Ile-77.
791673	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 645 as residues: Arg-72 to Glu-84.
792080	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 646 as residues: Asn-47 to Asp-53, Ser-75 to Ala-80.
793025	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 647 as residues: Glu-52 to Lys-58.
793043	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 648 as residues: Ser-1 to Gly-8, Ile-71 to Ala-83, Asp-91 to Arg-96.
793386	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 649 as residues: Gly-23 to Ala-56, Thr-58 to Ser-65, Gly-69 to Glu-140, Ser-158 to Gly-165, Thr-169 to Arg-175, Pro-181 to Glu-186.
795144	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 650 as residues: Arg-2 to Ser-14, Arg-18 to Glu-26, Ile-62 to Ser-72, Asp-132 to Asp-138, Thr-147 to Arg-163, Cys-180 to Asn-194, Asp-199 to Glu-205, Arg-212 to Leu-218, Thr-248 to Arg-270, Leu-278 to Ala-286, Gln-322 to Phe-329.
795911	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 651 as residues: His-4 to Asn-11.
795962	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 652 as residues: Pro-63 to Gly-71, Arg-96 to Gly-101, Phe-106 to Leu-111, Arg-124 to Met-130, Cys-154 to Ala-160, Glu-163 to Ser-169, Arg-180 to Phe-191.
796221	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 653 as residues: Leu-12 to Gly-41, Ser-54 to Gly-62, Trp-107 to Pro-113, Glu-140 to Lys-145, Leu-147 to Lys-155, Arg-177 to Asp-183, Glu-189 to Lys-197, Leu-202 to Thr-207, Leu-275 to Asp-283.
796283	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 654 as residues: Ser-1 to Ser-12, Pro-87 to Arg-92.
796392	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 655 as residues: Glu-16 to Gly-28, Glu-54 to Asp-75, Lys-92 to Lys-101, Tyr-106 to Glu-118, Glu-127 to Val-164, Ser-172 to Lys-185, Arg-199 to Phe-236, Arg-255 to Ser-262, Pro-265 to Glu-275.
797655	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 656 as residues: Pro-34 to Val-40, Pro-47 to Asp-58, Pro-60 to Leu-67, Ser-74 to Ala-81, Pro-99 to Arg-127, Thr-145 to Gln-155.
799486	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 657 as residues: Ala-9 to Asp-15, Trp-132 to Val-139.
800221	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 659 as residues: Asn-41 to Lys-53, Tyr-94 to Ile-99, Asp-123 to Thr-136.
800376	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 660 as residues: Arg-7 to Ala-14, Gln-80 to Ser-88, Val-96 to Gln-101, Lys-149 to Tyr-159, Gln-177 to Arg-185.
800567	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 661 as residues: Gly-1 to Ala-21, Ser-262 to Asn-274, Pro-277 to Cys-285, Pro-291 to Gly-303, Pro-310 to Gly-315, Pro-321 to Ala-326, Asn-334 to Ser-342, Gly-380 to Arg-386.
800652	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 662 as residues: Ser-15 to Gln-20, Asp-25 to Tyr-32, Phe-35 to Tyr-62, Arg-94 to Lys-102,



	Glu-137 to Phe-146, Phe-148 to Phe-158, Arg-166 to Gly-177, Met-180 to Asn-194, Arg-198 to Gln-212, Ala-236 to Glu-241, Val-243 to Glu-248.
800748	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 663 as residues: Glu-20 to Leu-33, Tyr-59 to Pro-69, Ala-92 to Asp-102, Leu-120 to Cys-129, Glu-143 to Tyr-148, Pro-168 to Leu-173, Asp-179 to Val-189, Thr-221 to Pro-228, Asp-249 to Ser-261, Thr-336 to Lys-342, Pro-377 to Asp-387, Arg-391 to Gly-397, Asp-428 to Ile-434, Asn-529 to Thr-559.
802032	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 664 as residues: Glu-74 to Trp-82.
802050	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 665 as residues: Ser-28 to Cys-38.
805551	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 666 as residues: Pro-6 to Tyr-19.
805662	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 667 as residues: Arg-29 to Ser-35, Ser-79 to Gly-91, Pro-105 to Arg-120, Thr-168 to Glu-175, Phe-187 to Ala-200, Arg-272 to Lys-282, Arg-325 to Asp-330, Arg-332 to Phe-338, Arg-358 to Lys-368, Cys-433 to Asn-441, Leu-456 to Asp-461.
805750	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 668 as residues: Glu-6 to Arg-13, Ser-53 to Gly-60, Arg-84 to Gln-90, Pro-101 to Thr-106.
805860	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 669 as residues: Thr-3 to Thr-8, Thr-55 to Ala-60.
805886	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 670 as residues: Trp-3 to Gly-16, Pro-19 to Ser-30, Gly-68 to Glu-74, Pro-81 to Lys-86, Ser-93 to Trp-98, Arg-102 to Asp-115, Arg-203 to Gly-210.
806706	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 671 as residues: Ser-10 to Ser-18, Arg-26 to Gly-33, Val-47 to Leu-60, Gly-79 to Phe-86, Gln-94 to Ser-99, Leu-126 to Cys-131.
811637	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 672 as residues: Arg-8 to Gly-20, Ala-27 to Ser-39, Gly-41 to Trp-55, Arg-58 to Gly-66, Asp-70 to Ser-88, Asp-108 to Tyr-117, Val-155 to Asn-164, Ile-168 to Lys-174, Lys-177 to Val-182, Pro-192 to Arg-200, Met-216 to Gly-225, Lys-232 to Val-237, Lys-261 to Arg-273, Ala-280 to Tyr-307.
812338	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 674 as residues: Pro-11 to Lys-18, Pro-25 to Ala-32, Gly-59 to Gly-64, Asn-73 to Phe-78.
812439	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 675 as residues: Leu-1 to Asn-7, Met-9 to Gln-14, His-25 to Gly-36, Phe-44 to Asp-49, Cys-61 to Cys-69, Gly-80 to Phe-85, Pro-91 to Gly-103, Asp-121 to Trp-128, Asp-130 to Ala-170, Trp-172 to Cys-183, Lys-193 to Asp-199, Pro-201 to Cys-210, Pro-217 to Asp-237, Thr-274 to Asn-280, Gly-292 to Cys-298, Asp-316 to Asp-326, Gly-339 to Asn-350.
812645	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 676 as residues: Tyr-99 to Glu-105, Gly-123 to His-139, Ile-148 to Glu-154.
812770	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 677 as residues: Ser-7 to Ser-16, Gln-41 to Ala-67, Glu-84 to Arg-91, Lys-98 to Gly-112, Arg-119 to Met-127, Glu-143 to Glu-149, Asp-161 to Ala-169, Ser-174 to Gln-182, Glu-197 to Glu-210, Lys-217 to Arg-224.
813080	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 679 as residues: Trp-47 to Val-55, Thr-142 to Ser-155.
815326	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 681 as residues: Thr-33 to Ile-38.
815740	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 682 as residues: Gln-35 to Ser-49.
824865	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 684 as residues: Arg-1 to Gln-13, Arg-33 to Arg-43, Lys-125 to Tyr-130, Ser-166 to Ser-171, Leu-212 to His-220.
825138	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 685 as



	residues: Gln-15 to Asn-31.
825535	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 686 as residues: His-6 to Asn-11, Asp-74 to Ala-83, Asp-95 to Leu-101, Leu-108 to Ser-113.
827046	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 688 as residues: His-28 to Asn-33.
827168	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 689 as residues: Met-4 to Lys-11, Pro-37 to Gly-44, Arg-136 to Gly-145, Pro-171 to Gly-181.
827195	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 690 as residues: Ser-1 to Gly-24.
827249	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 691 as residues: Leu-27 to Arg-32, Leu-81 to Pro-86, Pro-91 to Cys-111, His-122 to Asn-132, Pro-142 to Ile-148, Asp-156 to Gln-164, Gly-185 to Ser-190, Cys-203 to Gly-212, His-219 to Ser-230, Val-249 to Phe-255, Glu-276 to Ala-281, Pro-324 to Ser-331, Thr-341 to Val-346, Ala-370 to Gly-375.
827447	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 692 as residues: Leu-1 to Asp-15, Glu-48 to Lys-61, Thr-84 to Ile-92, Glu-108 to Glu-125, Lys-157 to Gln-164, Thr-166 to Glu-173.
827515	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 693 as residues: His-3 to Ile-11, Pro-14 to Asp-22.
827621	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 694 as residues: Gly-1 to His-7, Tyr-53 to Asn-60, Thr-80 to Gly-87, Lys-95 to Gly-102, Pro-129 to Thr-134.
827883	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 695 as residues: Gly-1 to Thr-13, Ser-69 to Trp-78, Cys-94 to Tyr-99.
828040	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 696 as residues: Gly-1 to Gln-10, Asn-18 to Lys-25, Gln-35 to Gly-40.
828360	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 697 as residues: Ser-11 to Ile-17, Asn-43 to Pro-48, Ser-64 to Trp-70.
828506	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 698 as residues: Pro-3 to His-8, Arg-24 to Leu-38.
828898	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 700 as residues: Gly-62 to Asn-77, Trp-118 to Glu-123, Asn-165 to Lys-172, Thr-225 to Asn-243, Phe-261 to Pro-267, Lys-300 to Trp-310, Asn-370 to Met-375.
828959	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 701 as residues: Lys-20 to Trp-26, Arg-41 to Gly-46.
829081	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 702 as residues: Lys-178 to Gly-184, Ile-186 to Asp-192, Pro-225 to Thr-234.
830069	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 703 as residues: Lys-20 to Asn-26, Lys-37 to Met-42, Cys-51 to Ser-57, Pro-59 to Cys-64, Gln-80 to Gly-87, Gln-98 to Glu-121, Phe-144 to Ser-149, Lys-158 to Val-169, Ser-171 to Pro-177, Lys-185 to Val-190, Glu-193 to Ser-201, Leu-209 to Gly-216, Cys-218 to Thr-224.
830109	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 704 as residues: Ser-1 to Gly-9.
830176	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 705 as residues: Gly-66 to Arg-74, Pro-87 to Arg-95, Glu-141 to Leu-149, Gln-225 to Ser-230, Pro-249 to Ile-256, Pro-351 to Gly-357.
830241	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 706 as residues: Pro-7 to Pro-17, Leu-20 to Gly-26, Leu-48 to Val-54, Pro-65 to Asn-70, Glu-90 to Ala-95, Ala-102 to Gln-116, Glu-122 to Leu-137, Val-183 to Leu-192, Ala-235 to Ile-256, Gly-264 to Asp-270, Phe-282 to Ile-288, Arg-309 to Ala-314, Asn-330 to Asp-336, Ala-338 to Asp-344, Lys-358 to Lys-367.
830402	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 708 as residues: Gln-8 to Ser-15, His-57 to Ser-64.

830414	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 709 as residues: Gly-1 to Arg-12, Ser-87 to Tyr-95, Arg-149 to Glu-155, Tyr-190 to Asp-195, Pro-265 to Leu-272, Ser-291 to Gly-305.
830444	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 710 as residues: Ser-47 to Tyr-54.
830476	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 711 as residues: Arg-5 to Leu-18, Thr-21 to Leu-29, Ile-32 to Ala-39, Glu-48 to Arg-56, Gln-62 to Trp-68, Glu-71 to Gly-78.
830624	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 712 as residues: Ala-21 to Pro-26, Arg-37 to Phe-44, Thr-67 to Lys-75, Ser-81 to Ser-86, Val-116 to Gln-127, Gly-192 to Thr-197, Trp-201 to Asn-207, Glu-243 to Ile-252, Lys-267 to Pro-273, Pro-292 to Phe-300, Lys-303 to Ala-314, Phe-344 to Asp-360, Ser-379 to Gly-386, Phe-389 to Asn-396, Glu-405 to Lys-413.
830643	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 713 as residues: Gly-10 to Gly-15, Val-59 to Lys-64, Lys-131 to Gly-140, Ala-220 to Asn-230, Gly-313 to Arg-321, His-331 to Thr-336, Pro-352 to Gly-359, Thr-361 to Cys-370.
830714	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 714 as residues: Glu-17 to Thr-24.
830826	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 715 as residues: Glu-14 to Ala-19, Arg-21 to Glu-28.
830888	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 716 as residues: Lys-63 to Asn-72, Arg-87 to Gly-92, Pro-125 to Gln-130.
830984	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 717 as residues: Thr-1 to Thr-10, Gly-29 to Gly-35, Leu-42 to Asp-64, Asp-71 to Ser-99, Gly-112 to Asp-132, Ser-178 to Ala-184.
831015	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 718 as residues: Gln-47 to Cys-53, Asn-66 to Cys-71, Arg-127 to Ala-141, Arg-143 to Lys-169, Lys-174 to Tyr-179.
831080	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 719 as residues: Tyr-10 to Asp-22, Pro-40 to Met-49.
831101	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 720 as residues: Asp-1 to Pro-10, Pro-39 to Trp-57, Ser-60 to Gly-68, Glu-105 to Pro-113, Thr-118 to Lys-124, Phe-131 to Tyr-139, Arg-153 to Lys-162, Lys-189 to Arg-194, Ala-230 to Ala-236, Trp-259 to Gln-266, Ala-272 to Tyr-277.
831146	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 721 as residues: Leu-23 to Asp-31, Gln-46 to Ile-69, Ile-120 to Lys-128, Pro-148 to Asp-154, Pro-216 to Val-223, Asn-261 to Ala-273.
831215	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 722 as residues: Gln-27 to Glu-52, Pro-92 to Pro-99, Asp-109 to Asp-116, Gln-123 to Ala-131, Leu-159 to His-164, Glu-176 to Val-183, Arg-195 to Glu-200, Gln-210 to Tyr-215, Glu-244 to Arg-255, Thr-262 to Asp-267, Pro-286 to Trp-292, Arg-302 to Asn-309, Gln-318 to Ser-323, Gln-341 to Ile-348, Lys-361 to Ile-374, Leu-378 to Gln-394.
831231	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 723 as residues: Lys-1 to Val-8, Ser-133 to Arg-139, Arg-163 to Leu-171, Arg-307 to Gln-320, Val-330 to Gly-335.
831242	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 724 as residues: Asn-55 to Pro-63, Arg-132 to Tyr-139, Phe-174 to Lys-183.
831291	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 727 as residues: Gly-1 to Gln-9, Asn-11 to Arg-16, Cys-28 to His-33, Pro-51 to Pro-57, Glu-66 to Glu-72, Pro-84 to Asp-89, Pro-104 to Asp-109, Glu-122 to Thr-132.
831382	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 728 as residues: Pro-13 to Pro-20, Thr-44 to His-49, Ala-72 to Phe-78.
831624	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 729 as residues: Ser-37 to Asp-43, Lys-266 to Ser-272, Glu-304 to Thr-318, Leu-345 to Ser-

	359, Gln-423 to Ala-439.
831640	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 730 as residues: Cys-16 to Ser-23.
831688	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 731 as residues: Pro-16 to Asp-23, Arg-48 to Glu-55, Gly-107 to Val-112, Glu-133 to Leu-140, Asn-163 to Gly-169, Gly-191 to Lys-196.
831690	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 732 as residues: Pro-36 to Trp-51, Arg-96 to Gly-104, Glu-134 to Asn-144, Pro-203 to His-210, Cys-228 to Asp-235, Gly-278 to Tyr-284, Ser-309 to Pro-316, Thr-325 to Ala-333, Ser-337 to Glu-357, Tyr-390 to Gly-403, Tyr-409 to Gly-421.
831718	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 733 as residues: Leu-64 to Arg-71, Leu-99 to Ser-105.
831832	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 734 as residues: Thr-8 to Ser-16.
831907	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 735 as residues: Leu-15 to Ser-20.
831938	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 736 as residues: Gly-74 to Val-79, Ser-94 to Arg-106, Asp-157 to Lys-162, Pro-354 to Gln-364, Arg-371 to Arg-385.
831954	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 737 as residues: Thr-48 to Ser-58, Gly-112 to Pro-129, Ala-156 to Ser-167.
832028	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 738 as residues: Lys-1 to Asn-14, Pro-103 to Tyr-111.
832043	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 739 as residues: Arg-9 to Val-17, Phe-110 to Lys-130, Gly-138 to Ala-143.
832055	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 740 as residues: Asp-1 to Lys-11, Ser-22 to Arg-32.
832124	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 741 as residues: Pro-10 to His-17, Thr-41 to Ala-51, Ser-60 to Pro-67, Leu-70 to Lys-78, Pro-95 to Ser-102, Ala-114 to Pro-122, Ile-125 to Pro-132, Glu-165 to Trp-172, Arg-194 to Gln-209.
832145	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 742 as residues: His-1 to Gln-7, Leu-11 to Glu-22, Gly-43 to Cys-52.
832254	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 743 as residues: Pro-1 to Ala-32, Phe-53 to Asp-59.
832331	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 744 as residues: Ser-6 to Asn-67, Gly-69 to Gly-98, Ser-100 to Phe-128.
832401	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 746 as residues: Gln-1 to Gly-6, Thr-9 to Asp-20, Met-22 to Asp-33, Pro-62 to Gly-70, Pro-79 to Lys-85, Asn-99 to Ser-104, Arg-154 to Glu-164.
832492	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 749 as residues: Cys-1 to Gly-6, Glu-27 to Leu-33, Lys-58 to Tyr-63, Glu-65 to Thr-79, Leu-83 to Lys-92.
832598	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 750 as residues: Ser-65 to Arg-79, Asn-81 to Leu-90, Ser-123 to Gly-135.
834510	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 752 as residues: Arg-83 to Lys-90, Arg-189 to Ser-195, Ser-197 to His-203.
835139	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 753 as residues: Ala-40 to Asn-49, Glu-76 to Ser-83, Cys-102 to Thr-113, Pro-143 to Thr-152, Gly-160 to Thr-177, Cys-204 to His-212.
835142	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 754 as residues: Pro-1 to Pro-6, Val-14 to Val-23, Tyr-124 to Lys-132, Gln-141 to Tyr-148, Glu-215 to Pro-221.
835271	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 755 as residues: Pro-1 to Pro-8, Asp-66 to Asn-78, Pro-81 to Ser-95, Thr-111 to Tyr-118, Asp-183 to Asn-188, Asp-190 to Asp-195, Cys-224 to Ile-232.

835369	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 756 as residues: Phe-65 to His-81, Thr-102 to Asp-117.
835430	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 757 as residues: Gln-48 to Lys-64, Glu-175 to Thr-183.
835462	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 758 as residues: Gly-8 to Gly-28, Glu-113 to Asn-122, Arg-144 to Gly-214, Ala-218 to Gly-232, Arg-243 to Glu-248.
835539	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 759 as residues: His-33 to Leu-39, Gly-49 to Glu-58, Ser-112 to Val-146.
835635	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 760 as residues: Leu-11 to Gly-47, Trp-61 to Ile-68, Glu-96 to Lys-103, Gly-110 to Gln-119, Ser-126 to Glu-160, Leu-172 to Ser-180, Thr-188 to Lys-193, Ser-197 to His-205, Gln-215 to Lys-227, Cys-299 to Asn-309, Lys-353 to Tyr-363, Trp-412 to Asp-418, Leu-448 to Leu-458, Gln-495 to Ser-503, Ser-587 to Thr-596, Ser-615 to Phe-620, Thr-653 to Asp-658, Glu-666 to Glu-671, Lys-710 to Gln-716.
836161	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 762 as residues: Ser-63 to Lys-71.
836213	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 763 as residues: Glu-9 to Gly-17.
836371	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 764 as residues: Ser-11 to Ser-31, Thr-39 to Trp-45, Ser-61 to Tyr-67, Asp-93 to Gln-100, Arg-124 to Asn-138, Val-141 to Asp-150.
837181	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 767 as residues: Ala-15 to Asp-34, Met-43 to Ser-48, Gln-80 to Glu-94, Glu-129 to Ser-135, Asp-139 to Ala-144, Glu-172 to Gln-179, Glu-266 to Ala-273.
837337	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 769 as residues: Tyr-29 to Asn-37.
837551	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 771 as residues: Arg-1 to Gly-7, Pro-9 to Ala-19.
837622	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 772 as residues: Gly-5 to Asp-26, Glu-62 to Phe-69.
839949	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 774 as residues: Ala-6 to Ser-16, Ser-36 to Gln-48, Pro-57 to Tyr-65, Glu-80 to Asn-87, Tyr-148 to Phe-153, Pro-177 to Asn-182, Ser-221 to Ser-229, Cys-252 to Asp-265, Tyr-386 to Tyr-393, Leu-427 to Trp-438, Leu-487 to Tyr-492.
840000	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 775 as residues: Gly-5 to Ser-21, Glu-30 to Glu-37, Glu-49 to Lys-57, Pro-92 to Arg-98, Leu-110 to Pro-118, Pro-223 to Pro-230, Ala-236 to Arg-241, Ser-285 to Gln-299, Leu-369 to Tyr-374.
840095	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 776 as residues: Pro-7 to Thr-13, Arg-25 to His-31, Gly-34 to His-40, Gln-127 to Asn-132, Asp-208 to Trp-214, Ser-243 to Phe-249, Glu-255 to Asp-261.
840166	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 777 as residues: Pro-1 to Ser-9.
840613	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 780 as residues: Pro-7 to Gly-14, Gln-31 to Tyr-37, Ile-87 to Ser-92, Gln-172 to Lys-184, Phe-197 to Asp-207, Leu-211 to Gln-225, Gln-297 to Lys-306, Glu-308 to Gly-318, Glu-420 to Gly-425, Arg-437 to Ala-447, Thr-507 to Asn-512, Ser-536 to Arg-541, Ser-634 to Gly-640, Lys-649 to Gln-656, Glu-661 to Leu-668, Tyr-709 to Gly-723, Gly-761 to Ala-767.
840699	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 781 as residues: Pro-20 to Gly-26, Pro-75 to Gly-80, Lys-92 to Thr-98, Thr-230 to Pro-239, His-249 to Met-254, Asp-304 to Arg-312.
840752	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 782 as residues: Val-25 to Ser-33.
840755	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 783 as

	residues: Pro-18 to Lys-25, Arg-28 to Cys-38, Val-61 to Leu-67, Pro-84 to Ser-95, Thr-174 to Gly-180, Thr-191 to Asn-197, Asp-205 to Pro-212, Lys-253 to Val-258, Lys-290 to Glu-297, Leu-299 to Cys-310.
841066	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 785 as residues: Gly-1 to Gly-21, Lys-38 to Thr-49, Leu-57 to Asp-62, Gln-74 to Phe-83, Gly-96 to Cys-109, Asn-124 to Gln-130, Glu-135 to Cys-140, Asp-149 to Lys-154, Tyr-164 to Asp-169, Pro-184 to Lys-192, Arg-209 to Ser-216, Asp-243 to Glu-250, Pro-329 to Glu-337, Thr-462 to Cys-471, Asn-481 to Thr-506.
841306	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 786 as residues: Arg-8 to Val-20, Glu-22 to Val-40, Glu-68 to Tyr-77, Lys-88 to Asp-95, Thr-116 to Lys-121.
842025	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 788 as residues: Glu-66 to Leu-74, Ile-116 to Glu-121, His-124 to Asp-129, Asn-152 to Tyr-157, Pro-171 to Asn-177, Glu-190 to Asn-201, Ile-215 to Gln-224, Lys-360 to Lys-370, Arg-389 to Asp-395, Glu-401 to Gly-415, Pro-431 to Cys-437.
842178	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 789 as residues: His-4 to Arg-9, His-16 to Gly-23.
842438	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 790 as residues: Ala-24 to Ser-32.
843289	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 791 as residues: His-55 to Arg-63, Glu-96 to Val-109, Ser-113 to Ala-119, Lys-140 to Tyr-150, Glu-157 to Ser-172, Gly-174 to Asn-185, Arg-223 to Pro-245, Leu-264 to Asp-272.
843447	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 792 as residues: Tyr-55 to Lys-64, Asp-80 to Trp-85.
843743	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 793 as residues: Pro-56 to Gly-63, Pro-70 to Asn-75, Gly-119 to Val-126, Trp-130 to Gly-137, Gln-210 to Glu-220, Lys-230 to Thr-236, Tyr-246 to Val-253.
843878	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 794 as residues: Thr-83 to Gly-88, Arg-144 to Pro-155, Arg-208 to Lys-215, Arg-286 to Gly-295.
844071	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 796 as residues: Thr-9 to Glu-15, Pro-20 to Gly-25, Arg-43 to Val-48, Pro-79 to Ala-91, Trp-114 to Glu-123, His-167 to Lys-177, Pro-179 to Arg-192, Asp-202 to Leu-208, Ala-261 to Asn-286, Gly-288 to Gly-296, Gly-301 to Met-308, Ser-343 to Asn-365, Phe-368 to Asn-379, Met-406 to Trp-413.
844444	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 797 as residues: Arg-9 to Thr-15, Pro-44 to His-50, Glu-62 to Arg-87, Glu-120 to Arg-126, Gln-144 to Asn-152, Ser-157 to Pro-169.
844561	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 798 as residues: Arg-30 to Ala-36, Gln-45 to Met-51.
844953	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 799 as residues: His-14 to Leu-25, Lys-104 to Ala-113.
844990	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 800 as residues: Ile-4 to Ala-15, Pro-39 to Gln-49, Lys-67 to Ser-72, Arg-87 to Leu-101, Thr-135 to Ser-146, Thr-177 to Val-183, Ser-185 to Ser-192, Ser-198 to Ala-216, Ser-221 to Pro-227, Val-242 to Gln-254, Ser-258 to Thr-266, Asn-274 to Arg-324.
845829	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 802 as residues: Asp-14 to Gly-29, Gln-88 to Asp-93, Glu-191 to Thr-196, Gly-262 to Ile-269.
HTAIR72R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 803 as residues: Arg-4 to Leu-11, Cys-18 to His-25.
HAPRM14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 805 as residues: Leu-16 to Ser-22, Lys-24 to Glu-38.
HMWEI22R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 806 as residues: Glu-1 to Thr-11, Pro-37 to Lys-42.

HMCGG09R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 812 as residues: Ser-2 to Ser-12, Gln-54 to His-61.
HFPDJ19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 814 as residues: Ile-118 to Lys-124.
HBGOI21R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 816 as residues: Asn-2 to Pro-9, Pro-17 to Leu-23, Asp-40 to Arg-61, Ala-90 to Ser-95, Ile-102 to Phe-108.
HCLCW23R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 817 as residues: Pro-1 to Trp-7, Pro-40 to Pro-45.
H2CAC11R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 819 as residues: Lys-7 to Thr-13, Asp-24 to Thr-30.
HOEMQ09R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 821 as residues: Thr-1 to Thr-6, Arg-13 to Ser-18.
HTLHA89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 825 as residues: Ser-1 to Asp-10, Ile-20 to Asp-26.
HWAFE43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 827 as residues: Pro-9 to Lys-16.
HTLIW74R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 829 as residues: Glu-67 to Gln-76, Lys-131 to Asp-136.
HDPTT19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 831 as residues: Asn-1 to Ser-8, His-37 to Pro-45.
HKBAC11R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 832 as residues: Gln-18 to Glu-23, Arg-43 to Arg-58.
HBGOU32R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 833 as residues: Arg-67 to Glu-74.
HNTNC82R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 834 as residues: Met-56 to Val-61, Pro-74 to Gly-91, Gly-112 to Pro-117.
HMCIB16R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 835 as residues: Gln-26 to Glu-37, Arg-42 to Gln-50, Ser-59 to Leu-74.
HAPNX90R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 836 as residues: Gly-1 to His-13.
HAJBZ28R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 838 as residues: Leu-8 to Gly-13, Glu-73 to Glu-81, Asn-88 to Arg-94.
HAGGW13R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 839 as residues: Pro-1 to Asp-10, Met-39 to Gly-45.
HAHDV81R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 840 as residues: Lys-2 to Arg-12.
HACBP41R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 841 as residues: Gly-1 to Phe-7, Arg-23 to Ser-29, Ser-34 to Ala-39, Lys-50 to Ile-56.
HESAN74R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 843 as residues: Gly-1 to Thr-9, Phe-28 to Lys-43.
HAPNU02R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 845 as residues: Pro-59 to Gly-75, Pro-84 to Gly-91, Cys-102 to Pro-114.
HOUGB18R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 846 as residues: Lys-7 to Thr-13, Ser-25 to Thr-30.
HBAGQ35R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 847 as residues: Leu-25 to Glu-32, Pro-42 to Gly-47, Cys-61 to Gly-68.
HAPQM68R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 849 as residues: Thr-44 to Lys-56, Arg-93 to Pro-99, Ser-104 to Pro-112.
HDPQN35R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 851 as residues: Pro-1 to Pro-6, Glu-31 to Asp-40.
HAPNU41R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 852 as residues: Glu-13 to Val-18.
HSYCT58R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 853 as residues: Arg-5 to Gly-14, Leu-34 to Arg-40, Leu-42 to Ala-49.
HFKLT54R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 854 as

	residues: Gln-51 to Tyr-58.
HTXNT90R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 855 as residues: Phe-121 to Asp-126.
H6BSD14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 856 as residues: Glu-6 to Glu-21.
HAPAK90R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 862 as residues: Glu-20 to Gly-26.
HAPBV57R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 865 as residues: Thr-8 to Leu-13.
HAPQO76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 867 as residues: Pro-10 to Thr-25, Pro-46 to Leu-55.
HBKDI63R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 869 as residues: Glu-8 to Asn-13, Arg-16 to Thr-29.
HCLCX30R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 871 as residues: Pro-47 to Trp-53, Ser-56 to Ser-66.
HDTFW96R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 873 as residues: Ser-41 to Lys-48.
HDTLW91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 874 as residues: Trp-12 to Ser-17.
HE9GW86R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 875 as residues: Pro-52 to Glu-59.
HFACI43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 876 as residues: Asn-4 to Glu-37.
HHFLJ48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 878 as residues: Arg-1 to Arg-6, Gly-26 to Ala-55.
HOEKC43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 882 as residues: Ala-1 to Ser-12, Thr-21 to Arg-31.
HPJCZ62R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 883 as residues: Lys-1 to Lys-12, Ala-16 to Ala-22, Gly-31 to Lys-36.
HSXEN17R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 885 as residues: Glu-1 to His-6, Gly-19 to Trp-31.
HMCGG17R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 886 as residues: Val-22 to Ser-28, Arg-62 to Ile-69.

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide sequence shown in SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by the cDNA in the related cDNA clone contained in a deposited library or encoded by a polynucleotide that hybridizes to the complement of an epitope encoding sequence of SEQ ID NO:X, or an epitope encoding sequence contained in the deposited cDNA clone under stringent hybridization conditions, or alternatively, under lower stringency hybridization conditions, as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to this complementary strand under stringent hybridization conditions or alternatively, under lower stringency hybridization conditions, as defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.



Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention, and immunogenic and/or antigenic epitope fragments thereof can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light

chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a  
5 disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

10 Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively,  
15 deleting the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See,  
20 D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine  
25 peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope

derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

5 Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., Proc. Natl. Acad. Sci. USA  
10 88:8972- 897 (1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> nitriloacetic acid-agarose column  
15 and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to  
20 modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol.  
25 Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA  
30 segments by homologous or site-specific recombination to generate variation in the

polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

As discussed herein, any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, polypeptides of the present invention which are shown to be secreted can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

In certain preferred embodiments, proteins of the invention comprise fusion proteins wherein the polypeptides are N and/or C- terminal deletion mutants. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions mutants. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell

or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

5

#### Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples

of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture  
5 mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and  
10 ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph,  
15 pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated  
20 transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

25 A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most

preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether  
5 directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition,  
10 polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed  
15 in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express polypeptides of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast  
20 which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using  $O_2$ . This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for  $O_2$ .  
25 Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., et al., *Yeast*



5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and

which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

10 In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if  
15 desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid,  
20 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L  
25 (levorotary).

Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see, e.g., Carter et al., *Nucl. Acids Res.* 13:4331 (1986); and Zoller et al., *Nucl. Acids Res.* 10:6487 (1982)), cassette mutagenesis (see, e.g., Wells et al., *Gene* 34:315

(1985)), restriction selection mutagenesis (*see, e.g., Wells et al., Philos. Trans. R. Soc. London SerA 317:415 (1986)*).

The invention additionally, encompasses polypeptides of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (*see U.S. Patent No. 4,179,337*). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between

about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release  
5 desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200; 500; 1000; 1500; 2000; 2500; 3000; 3500; 4000; 4500; 5000; 5500; 6000; 6500; 7000; 7500; 8000; 8500; 9000; 9500; 10,000;  
10 10,500; 11,000; 11,500; 12,000; 12,500; 13,000; 13,500; 14,000; 14,500; 15,000; 15,500; 16,000; 16,500; 17,000; 17,500; 18,000; 18,500; 19,000; 19,500; 20,000; 25,000; 30,000; 35,000; 40,000; 50,000; 55,000; 60,000; 65,000; 70,000; 75,000; 80,000; 85,000; 90,000; 95,000; or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure.  
15 Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconj. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

20 The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting  
25 pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues;  
30 those having a free carboxyl group may include aspartic acid residues glutamic acid

residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

5       As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine,  
10   histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may  
15   select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this  
20   moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for  
25   derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be  
30   attached to the protein either directly or by an intervening linker. Linkerless systems

for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated  
5 herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG,  
10 polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of  
15 different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-  
20 2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference.  
25 Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12,  
30 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of

substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The lung cancer antigen polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or an amino acid sequence encoded by SEQ ID NO:X, and/or an amino acid sequence encoded by the cDNA in a related cDNA clone contained in a deposited library (including fragments, variants, splice variants, and fusion proteins, corresponding to any one of these as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to

the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

- 5           Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers
- 10 of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides
- 15 of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, or contained in a polypeptide encoded by SEQ ID NO:X, and/or by the cDNA in the related cDNA clone contained in a deposited library). In one instance, the covalent associations are cross-linking between cysteine residues located within the
- 20 polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the
- 25 heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from
- 30 another protein that is capable of forming covalently associated multimers, such as for



example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention

containing Flag® polypeptide sequence. In a further embodiment, associations of proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

- 5       The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).
- 10   Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely.
- 15   modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the
- 20   polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

- Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained
- 25   in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the
- 30   invention to a sequence encoding a linker polypeptide and then further to a synthetic

polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described  
5 herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

10

### Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as  
15 determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id  
20 antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM,  
25 IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and  
30 fragments comprising either a VL or VH domain. Antigen-binding antibody

fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, or by size in contiguous amino acid residues. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog,

or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention  
5 bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor  
10 activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at  
15 least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing  
20 antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of  
25 the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No.  
30 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res.

58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups,

proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more  
5 non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited  
10 to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols,  
15 polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display  
20 technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by  
25 reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.



Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire

or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999

(1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody

libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

5 Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the  
10 human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous  
15 deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention.  
20 Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG,  
25 IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European  
30 Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825;

5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

#### *Polynucleotides Encoding Antibodies*

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a

polypeptide of the invention. preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody  
5 may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of  
10 the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized  
15 or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe  
20 specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the  
25 antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY  
30 and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley &

cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

*Example 34: High-Throughput Screening Assay Identifying Myeloid Activity*

10

The following protocol is used to assess myeloid activity of polypeptide of the present invention by determining whether polypeptide of the present invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 32, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675 uM  $\text{CaCl}_2$ . Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates

tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient  
5 cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required  
10 number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentacin, and 1% Pen-Strep. These cells are treated with supernatants containing  
15 polypeptide of the present invention or polypeptide of the present invention induced polypeptides as produced by the protocol described in Example 31.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one  
20 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100,000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly  
25 from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each  
30 well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 37. The plates containing the remaining treated



GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using Sall and  
5 NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 33-34.

Other constructs can be made using the above description and replacing GAS with a  
10 different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 35 and 36. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly,  
15 other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

*Example 33: High-Throughput Screening Assay for T-cell Activity.*

20

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to  
25 activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4<sup>+</sup> Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo  
30 vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 33-34, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5' : GCGCCTCGAGATTTCCCGAAATCTAGATTTCCCGAAATGATTTCCCGAAAT  
 10 GATTTCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:889)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:890)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5' : CTCGAGATTTCCCGAAATCTAGATTTCCCGAAATGATTTCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACT  
 TCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACT  
 CCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTG  
 20 ACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCC  
 AGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3'  
 (SEQ ID NO:891)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

30 The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the

394

Growth hormone family

	GH	?	-	+	-	5	
	PRL	?	+/-	+	-	1,3,5	
5	EPO	?	-	+	-	5	GAS(B-
	CAS>IRF1=IFP>>Ly6)						

Receptor Tyrosine Kinases

	EGF	?	+	+	-	1,3	GAS (IRF1)
10	PDGF	?	+	+	-	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)

393

	<u>Ligand</u>	<u>JAKs</u>				<u>STATS</u>	<u>GAS(elements) or ISRE</u>
		<u>tyk2</u>	<u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>		
	<u>IFN family</u>						
5	IFN-a/B	+	+	-	-	1,2,3	ISRE
	IFN-g		+	+	-	1	GAS
	(IRF1>Lys6>IFP)						
	IL-10	+	?	?	-	1,3	
10	<u>gp130 family</u>						
	IL-6 (Pleiotrohic)	+	+	+	?	1,3	GAS
	(IRF1>Lys6>IFP)						
	IL-11(Pleiotrohic)	?	+	?	?	1,3	
	OnM(Pleiotrohic)	?	+	+	?	1,3	
15	LIF(Pleiotrohic)	?	+	+	?	1,3	
	CNTF(Pleiotrohic)	-/+	+	+	?	1,3	
	G-CSF(Pleiotrohic)	?	+	?	?	1,3	
	IL-12(Pleiotrohic)	+	-	+	+	1,3	
20	<u>g-C family</u>						
	IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
	IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP
	>>Ly6)(IgH)						
	IL-7 (lymphocytes)	-	+	-	+	5	GAS
25	IL-9 (lymphocytes)	-	+	-	+	5	GAS
	IL-13 (lymphocyte)	-	+	?	?	6	GAS
	IL-15	?	+	?	+	5	GAS
	<u>gp140 family</u>						
30	IL-3 (myeloid)	-	-	+	-	5	GAS
	(IRF1>IFP>>Ly6)						
	IL-5 (myeloid)	-	-	+	-	5	GAS
	GM-CSF (myeloid)	-	-	+	-	5	GAS

IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many  
5 cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive  
10 in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH,  
15 G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:888)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate  
20 STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway; reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the  
25 Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolarity to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degree C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 33-40.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide of the present invention directly (e.g., as a secreted protein) or by polypeptide of the present invention inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

#### *Example 32: Construction of GAS Reporter Construct*

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to

150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

- Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on
- 5 PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degree C for 6 hours.
- 10 While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of  $\text{CaCl}_2$  (anhyd); 0.00130 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.050 mg/L of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ; 0.417 mg/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 311.80 mg/L of Kcl; 28.64 mg/L of  $\text{MgCl}_2$ ; 48.84 mg/L of  $\text{MgSO}_4$ ; 6995.50 mg/L of NaCl; 2400.0 mg/L of  $\text{NaHCO}_3$ ; 62.50 mg/L of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 71.02 mg/L of  $\text{Na}_2\text{HPO}_4$ ; .4320 mg/L of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-
- 15 Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine- $\text{H}_2\text{O}$ ; 6.65 mg/ml of L-Aspartic Acid; 29.56
- 20 mg/ml of L-Cystine-2HCL- $\text{H}_2\text{O}$ ; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL- $\text{H}_2\text{O}$ ; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine;
- 25 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2 $\text{H}_2\text{O}$ ; and 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17
- 30 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of

blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 31: Production Of Polypeptide of the Invention For High-Throughput Screening Assays*

10

The following protocol produces a supernatant containing polypeptide of the present invention to be tested. This supernatant can then be used in the Screening Assays described in Examples 33-42.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8-10, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add



To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>. HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90  $\mu$ l of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10  $\mu$ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer:  $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

- 5      Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..
- 10      The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 30: Suppression of TNF alpha-induced adhesion molecule expression by a Agonist or Antagonist of the Invention*

- 15      The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion
- 20      molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of
- 25      cytokines and growth factor participate in the modulation of the expression of these CAMs.

- Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.
- 30      The potential of an agonist or antagonist of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated or suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal  
5 and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck).  
10 The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically  
15 occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places.  
20 Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

25 Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor).  
30 Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca<sup>2+</sup> comparison.

perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with  
5 an agonist or antagonist of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

The studies described in this example tested activity of agonists or antagonists of the  
10 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

#### *Example 29: Lymphadema Animal Model*

15 The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of an agonist or antagonist of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and  
20 histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in  
25 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

30 Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel

wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch.

5 The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on

10 days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The agonist or antagonist of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups

15 received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes

20 between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the

25 differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

30 
$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

#### *B. Steroid Impaired Rat Model*

- 5 The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *An. Intern.* 10 *Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The
- 15 systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).
- 20 To demonstrate that an agonist or antagonist of the invention can accelerate the healing process, the effects of multiple topical applications of the agonist or antagonist on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.
- 25 Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to
- 30 the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.
- The wounding protocol is followed according to section A, above. On the day of

tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

10            
$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with an agonist or antagonist of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

20            Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

25            Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer served as a positive tissue control and human brain tissue is used as a negative tissue control. Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic  
5 (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use  
10 Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of  
15 the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days  
20 commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically  
25 using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

An agonist or antagonist of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

30 Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in



e) Treatment with an agonist or antagonist of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 28: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models*

A. *Diabetic db+/db+ Mouse Model.*

To demonstrate that an agonist or antagonist of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

5 *Example 26: The Effect of Agonists or Antagonists of the Invention on the Growth of Vascular Endothelial Cells*

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2-5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16  
10 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. An agonist or antagonist of the invention, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

15 An increase in the number of HUVEC cells indicates that the compound of the invention may proliferate vascular endothelial cells, while a decrease in the number of HUVEC cell indicates that the compound of the invention inhibits vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity  
20 of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

*Example 27: Rat Corneal Wound Healing Model*

This animal model shows the effect of an agonist or antagonist of the invention on  
25 neovascularization. The experimental protocol includes:

- a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.
- b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.
- 30 c) Making a pocket (its base is 1-1.5 mm from the edge of the eye).
- d) Positioning a pellet, containing 50ng- 5ug of an agonist or antagonist of the invention, within the pocket.

dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

- 5 It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. 10 Neuroscience, 1990).

- Based on the data with FGF-2, agonists or antagonists of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The 15 potential effect of an agonist or antagonist of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 20 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

- 25 Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if an agonist or antagonist of the invention acts to prolong the survival of 30 dopaminergic neurons, it would suggest that the agonist or antagonist may be involved in Parkinson's Disease.

The studies described in this example tested activity of agonists or antagonists of the

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or agonists or antagonists of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without agonists or antagonists of the invention IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or agonists or antagonists of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with agonists or antagonists of the invention.

#### Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in

macrophages, a standard curve of a  $H_2O_2$  solution of known molarity is performed for each experiment.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

*Example 25: Biological Effects of Agonists or Antagonists of the Invention*

10 Astrocyte and Neuronal Assays.

Agonists or antagonists of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate an agonist or antagonist of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of an agonist or antagonist of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

30

Fibroblast and endothelial cell assays.

when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5  $\mu$ g/ml, and then incubated at room temperature for 5 minutes before FACSscan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml with increasing concentrations of agonists or antagonists of the invention and under the same conditions, but in the absence of agonists or antagonists. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of agonist or antagonist of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e. g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well. Increasing concentrations of agonists or antagonists of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20  $\mu$ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of  $H_2O_2$  produced by the

and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ( $10^6$ /ml) are treated with increasing concentrations of agonists or antagonists of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using  
5 commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules,  
10 molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

15 FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of agonists or antagonists of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are  
20 analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine  
25 whether a molecule of the invention functions as an inhibitor or activator of monocytes. Agonists or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow  
30 centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability

ul of medium containing 0.5  $\mu$ Ci of  $^3\text{H}$ - thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of  $^3\text{H}$ -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of agonists or antagonists of the invention.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

10.

*Example 24: Effect of Agonists or Antagonists of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells*

15 Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as  $\text{TNF-}\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FCyRII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

20 FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of agonist or antagonist of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

30 Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T



and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with agonists or antagonists of the invention identify the results of the activity of the agonists or antagonists on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with agonist or antagonist is used to indicate whether the agonists or antagonists specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice. Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and agonists or antagonists-treated mice.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

#### *Example 23: T Cell Proliferation Assay*

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of <sup>3</sup>H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 µl/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1 µg/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 10<sup>4</sup>/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of agonists or antagonists of the invention (total volume 200 µl). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100 µl of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100

various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

**In Vitro Assay-** Agonists or antagonists of the invention can be assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the agonists or antagonists of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$  M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with  $^3$ H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

**In Vivo Assay-** BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of agonists or antagonists of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues

sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

#### *Example 22: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation*

25

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with

30

*Example 20: Knock-Out Animals*

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (*e.g.*, knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (*i.e.*, animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (*e.g.*, lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, *e.g.*, by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding

endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic  
5 animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos;  
10 gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene  
15 transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of  
20 nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campbell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals or chimeric. The transgene may be integrated as a single transgene or as  
25 multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those  
30 of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the

aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

#### *Example 19: Transgenic Animals*

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment,

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an



the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

- 5       The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

*Example 18: Method of Treatment Using Gene Therapy - In Vivo*

10

- Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., *Cardiovasc. Res.* 35(3):470-479 (1997); Chao et al., *Pharmacol. Res.* 35(6):517-522 (1997); Wolff, *Neuromuscul. Disord.* 7(5):314-318 (1997); Schwartz et al., *Gene Ther.* 3(5):405-411 (1996); Tsurumi et al., *Circulation* 94(12):3281-3290 (1996) (incorporated herein by reference).

- 25       The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

- 30       The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) *Ann. NY Acad. Sci.* 772:126-139 and Abdallah B. et al. (1995) *Biol. Cell* 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase  
5 fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub>HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells  
10 resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately  $3 \times 10^6$  cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the  
15 invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a  
20 HindIII site at the 3' end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The  
25 final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately  $1.5 \times 10^6$  cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving  
30 cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and

*the Invention*

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued  
5 June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

10 Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting  
15 sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

20 The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then  
25 purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

30 Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the

and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

*Example 17: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of*

dosing scheme, based on administration and formulation, are provided in Example 13.

*Example 15: Method of Treating Increased Levels of the Polypeptide*

5       The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

10       In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well  
15       tolerated. The formulation of the antisense polynucleotide is provided in Example 13.

*Example 16: Method of Treatment Using Gene Therapy-Ex Vivo*

20       One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and  
25       the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer  
30       is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII

Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are  
5 incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

10 In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

15 In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

*Example 14: Method of Treating Decreased Levels of the Polypeptide*

20

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated  
25 that conditions caused by a decrease in the standard or normal expression level of a polypeptide of the present invention in an individual can be treated by administering the agonist or antagonist of the present invention. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the agonist or  
30 antagonist to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the agonist or antagonist for six consecutive days. The exact details of the

sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g.,  
5 dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment,  
10 Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13,  
15 IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in  
20 combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317;  
25 Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2  
30 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial

combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazolè, and tenidap.

In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate



CLARITHROMYCIN™ , and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in

nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

15 In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™,

20  
25  
30

lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with  
5 other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or  
10 concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

15 In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L,  
20 CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-1BB,  
25 TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International  
30 Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-

injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml  
5 of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention.  
10 Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

15 The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific  
20 embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to,  
25 vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or  
30 concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic

"Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and  
5 infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an  
10 emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., *J. Biomed. Mater.*  
15 *Res.* 15:167-277 (1981), and Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., *Id.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see generally*, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler  
20 (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci.(USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily,  
25 the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987);  
30 Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with  
5 a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to  
10 practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1 ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to  
15 therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be  
20 employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable  
25 carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release  
30 systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray.

deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

*Example 12: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample*

5

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

10

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

15

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

20

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

25

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

*Example 13: Formulation*

30

The invention also provides methods of treatment and/or prevention of diseases or



*Polynucleotide*

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a  
5 template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X; and/or the nucleotide sequence of the related cDNA in the cDNA clone contained in a deposited library. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

10 PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

15 PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

20 Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

25 Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image  
30 collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions,

and 25 µg kanamycin/ml (2xTY-AMP- KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 1013 transducing units/ml (ampicillin-resistant clones).

*Panning of the Library.* Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 1013 TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

*Characterization of Binders.* Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

*Example 11: Method of Determining Alterations in a Gene Corresponding to a*

antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al.,  
5 U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

**b) Isolation Of Antibody Fragments Directed Against Polypeptide of the Present  
10 Invention From A Library Of scFvs**

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide of the present invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

15 *Rescue of the Library.* A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-  
20 AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication  
25 WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III  
30 protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml

contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide of the present invention are prepared using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide of the present invention or, more preferably, with a secreted polypeptide of the present invention-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide of the present invention.

Alternatively, additional antibodies capable of binding to polypeptide of the present invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide of the present invention-specific antibody can be blocked by polypeptide of the present invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide of the present invention-specific antibody and are used to immunize an animal to induce formation of further polypeptide of the present invention-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such

vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

- 5 If the naturally occurring signal sequence is used to produce the polypeptide of the present invention, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

- 10 GGGATCCGGAGCCCAAATCTTCTGACAAACTCACACATGCCACCGTGCCAG  
CACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGA  
CACCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGTGGACGTAAGC  
CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT  
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC  
15 AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC  
AAGGTCTCCAACAAAGCCCTCCCAACCCCCATCGAGAAAACCATCTCCAAAGCC  
AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAG  
CTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGC  
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGAC  
20 CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC  
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT  
GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT  
GAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:887)

25 *Example 10: Production of an Antibody from a Polypeptide*

a) Hybridoma Technology

- The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing  
30 polypeptide of the present invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide of the present invention is prepared and purified to render it substantially free of natural

hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM).

- 5 Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200  $\mu$ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

10

#### *Example 9: Protein Fusions*

- The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

- 30 For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the

integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 or pC4 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in

*Example 8: Expression of a Polypeptide in  
Mammalian Cells*

The polypeptide of the present invention can be expressed in a mammalian cell. A typical  
5 mammalian expression vector contains a promoter element, which mediates the initiation of  
transcription of mRNA, a protein coding sequence, and signals required for the termination  
of transcription and polyadenylation of the transcript. Additional elements include  
enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites  
for RNA splicing. Highly efficient transcription is achieved with the early and late promoters  
10 from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI  
and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also  
be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include,  
for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat  
15 (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12M1 (ATCC 67109), pCMVSPORT 2.0, and  
pCMVSPORT 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and  
Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells,  
mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the  
20 polynucleotide integrated into a chromosome. The co-transfection with a selectable marker  
such as DHFR, gpt, neomycin, hygromycin allows the identification and isolation of the  
transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded  
protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that  
25 carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt,  
F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et  
Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-  
68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy  
et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175  
30 (1992). Using these markers, the mammalian cells are grown in selective medium and the  
cells with the highest resistance are selected. These cell lines contain the amplified gene(s)



Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One  $\mu\text{g}$  of BaculoGold™ virus DNA and 5  $\mu\text{g}$  of the plasmid are mixed in a sterile well of a microtiter plate containing 50  $\mu\text{l}$  of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10  $\mu\text{l}$  Lipofectin plus 90  $\mu\text{l}$  Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu\text{l}$  of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4° C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu\text{Ci}$  of  $^{35}\text{S}$ -methionine and 5  $\mu\text{Ci}$   $^{35}\text{S}$ -cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

5 Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

10 Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon, is amplified using the PCR protocol described in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard  
15 methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with  
20 appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

25 The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the  
30 cloned fragment is confirmed by DNA sequencing.

Five  $\mu$ g of a plasmid containing the polynucleotide is co-transfected with 1.0  $\mu$ g of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA",

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16  $\mu\text{m}$  membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5  $\mu\text{g}$  of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

#### *Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System*

25

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under

according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

- 5           The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

*Example 6: Purification of a Polypeptide from an Inclusion Body*

- 10           The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

          Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm  
15 (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

          The cells are then lysed by passing the solution through a microfluidizer  
20 (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

          The resulting washed inclusion bodies are solubilized with 1.5 M guanidine  
25 hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

          Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes  
30 of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilotri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated

cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR  
5 fragment in the particular somatic cell hybrid.

*Example 5: Bacterial Expression of a Polypeptide*

A polynucleotide encoding a polypeptide of the present invention is amplified using  
10 PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial  
15 expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is  
20 ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is  
25 isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto  
30 pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

**Example 3: Tissue specific expression analysis**

The Human Genome Sciences, Inc. (HGS) database is derived from sequencing tissue specific cDNA libraries. Libraries generated from a particular tissue are selected and the specific tissue expression pattern of EST groups or assembled contigs within these libraries is determined by comparison of the expression patterns of those groups or contigs within the entire database. ESTs which show tissue specific expression are selected.

The original clone from which the specific EST sequence was generated, is obtained from the catalogued library of clones and the insert amplified by PCR using methods known in the art. The PCR product is denatured then transferred in 96 well format to a nylon membrane (Schleicher and Scheull) generating an array filter of tissue specific clones. Housekeeping genes, maize genes, and known tissue specific genes are included on the filters. These targets can be used in signal normalization and to validate assay sensitivity. Additional targets are included to monitor probe length and specificity of hybridization.

Radioactively labeled hybridization probes are generated by first strand cDNA synthesis per the manufacturer's instructions (Life Technologies) from mRNA/RNA samples prepared from the specific tissue being analyzed. The hybridization probes are purified by gel exclusion chromatography, quantitated, and hybridized with the array filters in hybridization bottles at 65°C overnight. The filters are washed under stringent conditions and signals are captured using a Fuji phosphorimager.

Data is extracted using AIS software and following background subtraction, signal normalization is performed. This includes a normalization of filter-wide expression levels between different experimental runs. Genes that are differentially expressed in the tissue of interest are identified and the full length sequence of these clones is generated.

**Example 4: Chromosomal Mapping of the Polynucleotides**

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute

limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

5 Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full  
10 length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the  
15 RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis  
20 using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

25

#### *Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide*

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the sequence corresponding to SEQ ID NO:X, according to the method  
30 described in Example 1. (See also, Sambrook.)



Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 5. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to the nucleotide sequence of SEQ ID NO:X.

- 5 Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid  
10 mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using  
15 Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

- Alternatively, two primers of 17-20 nucleotides derived from both ends of the  
20 nucleotide sequence of SEQ ID NO:X are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25  $\mu\text{l}$  of reaction mixture with 0.5  $\mu\text{g}$  of the above cDNA template. A convenient reaction mixture is 1.5-5 mM  $\text{MgCl}_2$ , 0.01% (w/v) gelatin, 20  $\mu\text{M}$  each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of  
25 Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA  
30 product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HCBM HCBN HCBO	Breast, Cancer: (4004943 A5)	pSport 1	LP023
HNBT HNBH HNBV	Breast, Normal: (4005522B2)	pSport 1	LP023
HBCP HBCQ	Breast, Cancer: (4005522 A2)	pSport 1	LP023
HBCJ	Breast, Cancer: (9806C012R)	pSport 1	LP023
HSAM HSAN	Stromal cells 3.88	pSport 1	LP023
HVCA HVCB HVCC HVCD	Ovary, Cancer: (4004332 A2)	pSport 1	LP023
HSCK HSEN HSEO	Stromal cells (HBM3.18)	pSport 1	LP023
HSCP HSCQ	stromal cell clone 2.5	pSport 1	LP023
HUXA	Breast Cancer: (4005385 A2)	pSport 1	LP023
HCOM HCON HCOO HCOP HCOQ	Ovary, Cancer (4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	pSport 1	LP023
HBNM	Breast, Cancer: (9802C020E)	pSport 1	LP023
HVVA HVVB HVVC HVVD HVVE	Human Bone Marrow, treated	pSport 1	LP023

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HNSM	Stomach Tumour	pSport 1	LP020
HNLA	Liver Normal Met5No	pSport 1	LP020
HUTA	Liver Tumour Met 5 Tu	pSport 1	LP020
HOCN	Colon Normal	pSport 1	LP020
HOCT	Colon Tumor	pSport 1	LP020
HTNT	Tongue Tumour	pSport 1	LP020
HLXN	Larynx Normal	pSport 1	LP020
HLXT	Larynx Tumour	pSport 1	LP020
HTYN	Thymus	pSport 1	LP020
HPLN	Placenta	pSport 1	LP020
HTNG	Tongue Normal	pSport 1	LP020
HZAA	Thyroid Normal (SDCA2 No)	pSport 1	LP020
HWES	Thyroid Thyroiditis	pSport 1	LP020
HFHD	Ficollled Human Stromal Cells, 5Fu treated	pTrip1Ex2	LP021
HFHM,HFHN	Ficollled Human Stromal Cells, Untreated	pTrip1Ex2	LP021
HPCI	Hep G2 Cells, lambda library	lambda Zap-CMV XR	LP021
HBCA,HBCB,HBCC	H. Lymph node breast Cancer	Uni-ZAP XR	LP021
HCOK	Chondrocytes	pSPORT1	LP022
HDCA, HDCB, HDCC	Dendritic Cells From CD34 Cells	pSPORT1	LP022
HDMA, HDMB	CD40 activated monocyte dendritic cells	pSPORT1	LP022
HDDM, HDDN, HDDO	LPS activated derived dendritic cells	pSPORT1	LP022
HPCR	Hep G2 Cells, PCR library	lambda Zap-CMV XR	LP022
HAAA, HAAB, HAAC	Lung, Cancer (4005313A3): Invasive Poorly Differentiated Lung Adenocarcinoma	pSPORT1	LP022
HIPA, HIPB, HIPC	Lung, Cancer (4005163 B7): Invasive, Poorly Diff. Adenocarcinoma, Metastatic	pSPORT1	LP022
HOOH, HOOI	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot	pSPORT1	LP022
HIDA	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HUJA,HUJB,HUJC,HUJD,HUJE	B-Cells	pCMVSPORT 3.0	LP022
HNOA,HNOB,HNOC,HNOD	Ovary, Normal: (9805C040R)	pSPORT1	LP022
HNLM	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HSCL	Stromal Cells	pSPORT1	LP022
HAAX	Lung, Cancer: (4005313 A3) Invasive Poorly-differentiated Metastatic lung adenocarcinoma	pSPORT1	LP022
HUUA,HUUB,HUUC,HUUD	B-cells (unstimulated)	pTrip1Ex2	LP022
HWWA,HWWB,HWWC,HWWD,HWE,HWWF,HWWG	B-cells (stimulated)	pSPORT1	LP022
HCCC	Colon, Cancer: (9808C064R)	pCMVSPORT 3.0	LP023
HPDO HPDP HPDQ HPDR HPD	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma	pSport 1	LP023
HPCO HPCP HPCQ HPCT	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	pSport 1	LP023
HOCM HOCO HOCP HOCQ	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	pSport 1	LP023

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HHAS	CHME Cell Line	Uni-ZAP XR	LP014
HAJR	Larynx normal	pSport 1	LP014
HWLE HWLF HWLG HWLH	Colon Normal	pSport 1	LP014
HCRM HCRN HCRO	Colon Carcinoma	pSport 1	LP014
HWLI HWLJ HWLK	Colon Normal	pSport 1	LP014
HWLQ HWLR HWLS HWLT	Colon Tumor	pSport 1	LP014
HBFM	Gastrocnemius Muscle	pSport 1	LP014
HBOD HBOE	Quadriceps Muscle	pSport 1	LP014
HBKD HBKE	Soleus Muscle	pSport 1	LP014
HCCM	Pancreatic Langerhans	pSport 1	LP014
HWGA	Larynx carcinoma	pSport 1	LP014
HWGM HWGN	Larynx carcinoma	pSport 1	LP014
HWLA HWLB HWLC	Normal colon	pSport 1	LP014
HWLM HWLN	Colon Tumor	pSport 1	LP014
HVAM HVAN HVAO	Pancreas Tumor	pSport 1	LP014
HWGQ	Larynx carcinoma	pSport 1	LP014
HAQM HAQN	Salivary Gland	pSport 1	LP014
HASM	Stomach; normal	pSport 1	LP014
HBCM	Uterus; normal	pSport 1	LP014
HCDM	Testis; normal	pSport 1	LP014
HDJM	Brain; normal	pSport 1	LP014
HEFM	Adrenal Gland, normal	pSport 1	LP014
HBAA	Rectum normal	pSport 1	LP014
HFDM	Rectum tumour	pSport 1	LP014
HGAM	Colon, normal	pSport 1	LP014
HHMM	Colon, tumour	pSport 1	LP014
HCLB HCLC	Human Lung Cancer	Lambda Zap II	LP015
HLRA	L1 Cell line	ZAP Express	LP015
HHAM	Hypothalamus, Alzheimer's	pCMVSPORT 3.0	LP015
HKBA	Ku 812F Basophils Line	pSport 1	LP015
HS2S	Saos2, Dexamethosone Treated	pSport 1	LP016
HASA	Lung Carcinoma A549 TNFalpha activated	pSport 1	LP016
HTFM	TF-1 Cell Line GM-CSF Treated	pSport 1	LP016
HYAS	Thyroid Tumour	pSport 1	LP016
HUTS	Larynx Normal	pSport 1	LP016
HXOA	Larynx Tumor	pSport 1	LP016
HEAH	Ea.hy.926 cell line	pSport 1	LP016
HINA	Adenocarcinoma Human	pSport 1	LP016
HRMA	Lung Mesothelium	pSport 1	LP016
HLCL	Human Pre-Differentiated Adipocytes	Uni-Zap XR	LP017
HS2A	Saos2 Cells	pSport 1	LP020
HS2I	Saos2 Cells; Vitamin D3 Treated	pSport 1	LP020
HUCM	CHME Cell Line, untreated	pSport 1	LP020
HEPN	Aryepiglottis Normal	pSport 1	LP020
HPSN	Sinus Piniformis Tumour	pSport 1	LP020
HNSA	Stomach Normal	pSport 1	LP020

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HSFA	Human Fibrosarcoma	Uni-ZAP XR	LP013
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP013
HTRA	Human Trachea Tumor	Uni-ZAP XR	LP013
HE2A HE2D HE2E HE2H HE2I	12 Week Old Early Stage Human	Uni-ZAP XR	LP013
HE2B HE2C HE2F HE2G HE2P	12 Week Old Early Stage Human, II	Uni-ZAP XR	LP013
HNEA HNEB HNEC HNED HNEE	Human Neutrophil	Uni-ZAP XR	LP013
HBGA	Human Primary Breast Cancer	Uni-ZAP XR	LP013
HPTS HPTT HPTU	Human Pituitary, subtracted	Uni-ZAP XR	LP013
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP013
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP013
HTOA HTOD HTOE HTOF HTOG	human tonsils	Uni-ZAP XR	LP013
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP013
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP013
HOQB	Human OB HOS treated (1 nM E2) fraction I	Uni-ZAP XR	LP013
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP013
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP013
HROA HROC	HUMAN STOMACH	Uni-ZAP XR	LP013
HBJA HBJB HBJC HBJD HBJE	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP013
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP013
HCPA	Corpus Callosum	Uni-ZAP XR	LP013
HSOA	stomach cancer (human)	Uni-ZAP XR	LP013
HERA	SKIN	Uni-ZAP XR	LP013
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP013
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP013
HWTA HWTB HWTC	wilm's tumor	Uni-ZAP XR	LP013
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP013
HAPN HAPO HAPP HAPQ HAPR	Human Adult Pulmonary;re-excision	Uni-ZAP XR	LP013
HLTG HLTH	Human T-cell lymphoma;re-excision	Uni-ZAP XR	LP013
HAHC HAHD HAHE	Human Adult Heart;re-excision	Uni-ZAP XR	LP013
HAGA HAGB HAGC HAGD HAGE	Human Amygdala	Uni-ZAP XR	LP013
HSJA HSJB HSJC	Smooth muscle-ILb induced	Uni-ZAP XR	LP013
SHSA HSHB HSHC	Smooth muscle, IL1b induced	Uni-ZAP XR	LP013
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP013
HPJA HPJB HPJC	LNCAP prostate cell line	Uni-ZAP XR	LP013
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP013
HBTA	Bone Marrow Stroma, TNF&LPS ind	Uni-ZAP XR	LP013
HMCF HMCB HMCH HMCJ HMCJ	Macrophage-oxLDL; re-excision	Uni-ZAP XR	LP013
HAGG HAGH HAGI	Human Amygdala;re-excision	Uni-ZAP XR	LP013
HACA	H. Adipose Tissue	Uni-ZAP XR	LP013
HKFB	K562 + PMA (36 hrs),re-excision	ZAP Express	LP013
HCWT HCWU HCWV	CD34 positive cells (cord blood),re-ex	ZAP Express	LP013
HBWA	Whole brain	ZAP Express	LP013
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP013
HAVM	Temporal cortex-Alzheimer	pT-Adv	LP014
HAVT	Hippocampus, Alzheimer Subtracted	pT-Adv	LP014

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HMEB	Human microvascular Endothelial cells, fract. B	Lambda ZAP II	LP013
HUSH	Human Umbilical Vein Endothelial cells, fract. A, re-excision	Lambda ZAP II	LP013
HLQC HLQD	Hepatocellular tumor, re-excision	Lambda ZAP II	LP013
HTWJ HTWK HTWL	Resting T-cell, re-excision	Lambda ZAP II	LP013
HF6S	Human Whole 6 week Old Embryo (II), sub1	pBluescript	LP013
HHPS	Human Hippocampus, subtracted	pBluescript	LP013
HLIS	LNCAP, differential expression	pBluescript	LP013
HLHS HLHT	Early Stage Human Lung, Subtracted	pBluescript	LP013
HSUS	Supt cells, cyclohexamide treated, subtracted	pBluescript	LP013
HSUT	Supt cells, cyclohexamide treated, differentially expressed	pBluescript	LP013
HSDS	H. Striatum Depression, subtracted	pBluescript	LP013
HPTZ	Human Pituitary, Subtracted VII	pBluescript	LP013
HSDX	H. Striatum Depression, sub1 II	pBluescript	LP013
HSDZ	H. Striatum Depression, sub1	pBluescript	LP013
HPBA HPBB HPBC HPBD HPBE	Human Pineal Gland	pBluescript SK-	LP013
HRTA	Colorectal Tumor	pBluescript SK-	LP013
HSBA HSBB HSBC HSBM	HSC172 cells	pBluescript SK-	LP013
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBluescript SK-	LP013
HJBA HJBB HJBC HJBD	Jurkat T-cell, S1 phase	pBluescript SK-	LP013
HTNA HTNB	Human Thyroid	pBluescript SK-	LP013
HAHA HAHB	Human Adult Heart	Uni-ZAP XR	LP013
HE6A	Whole 6 week Old Embryo	Uni-ZAP XR	LP013
HFCA HFCB HFCC HFCD HFCE	Human Fetal Brain	Uni-ZAP XR	LP013
HFKE HFKE HFKE HFKE HFKE	Human Fetal Kidney	Uni-ZAP XR	LP013
HGBA HGBD HGBE HGBF HGBG	Human Gall Bladder	Uni-ZAP XR	LP013
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP013
HTEA HTEB HTEC HTEC HTEC	Human Testes	Uni-ZAP XR	LP013
HTTA HTTB HTTC HTTD HTTE	Human Testes Tumor	Uni-ZAP XR	LP013
HYBA HYBB	Human Fetal Bone	Uni-ZAP XR	LP013
HFLA	Human Fetal Liver	Uni-ZAP XR	LP013
HHFB HHFC HHFD HHFE HHFF	Human Fetal Heart	Uni-ZAP XR	LP013
HUVB HUVB HUVB HUVB	Human Umbilical Vein, End. remake	Uni-ZAP XR	LP013
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP013
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP013
HTAA HTAB HTAC HTAD HTAE	Human Activated T-cells	Uni-ZAP XR	LP013
HFEA HFEB HFEC	Human Fetal Epithelium (skin)	Uni-ZAP XR	LP013
HJPA HJPB HJPC HJPD	Human Jurkat Membrane Bound Polysomes	Uni-ZAP XR	LP013
HESA	Human Epithelioid Sarcoma	Uni-ZAP XR	LP013
HALS	Human Adult Liver, Subtracted	Uni-ZAP XR	LP013
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP013
HCAA HCAB HCAC	Cem cells, cyclohexamide treated	Uni-ZAP XR	LP013
HRGA HRGB HRGC HRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP013
HE9A HE9B HE9C HE9D HE9E	Nine Week Old Early Stage Human	Uni-ZAP XR	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HTJM	Human Tonsils, Lib 2	pCMVSPORT 2.0	LP012
HAMF HAMG	KMH2	pCMVSPORT 3.0	LP012
HABA HAJB HAJC	L428	pCMVSPORT 3.0	LP012
HWBA HWBB HWBC HWBD HWBE	Dendritic cells, pooled	pCMVSPORT 3.0	LP012
HWAA HWAB HWAC HWAD HWAE	Human Bone Marrow, treated	pCMVSPORT 3.0	LP012
HYAA HYAB HYAC	B Cell lymphoma	pCMVSPORT 3.0	LP012
HWHG HWHH HWHI	Healing groin wound, 6.5 hours post incision	pCMVSPORT 3.0	LP012
HWHF HWHG HWHH	Healing groin wound; 7.5 hours post incision	pCMVSPORT 3.0	LP012
HARM	Healing groin wound - zero hr post-incision (control)	pCMVSPORT 3.0	LP012
HBIM	Olfactory epithelium; nasalcavity	pCMVSPORT 3.0	LP012
HWDA	Healing Abdomen wound; 70&90 min post incision	pCMVSPORT 3.0	LP012
HWEA	Healing Abdomen Wound:15 days post incision	pCMVSPORT 3.0	LP012
HWJA	Healing Abdomen Wound:21&29 days	pCMVSPORT 3.0	LP012
HNAL	Human Tongue, frac 2	pSport1	LP012
HMJA	H. Meningima, M6	pSport1	LP012
HMKA HMKB HMKC HMKD HMKE	H. Meningima, M1	pSport1	LP012
HOFA	Ovarian Tumor I, OV5232	pSport1	LP012
HCFA HCFB HCFC HCFD	T-Cell PHA 16 hrs	pSport1	LP012
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport1	LP012
HMM A HMMB HMMC	Spleen metastatic melanoma	pSport1	LP012
HTDA	Human Tonsil, Lib 3	pSport1	LP012
HDBA	Human Fetal Thymus	pSport1	LP012
HDDA	Pericardium	pSport1	LP012
HBZA	Prostate,BPH, Lib 2	pSport1	LP012
HWCA	Larynx tumor	pSport1	LP012
HWKA	Normal lung	pSport1	LP012
HSMB	Bone marrow stroma,treated	pSport1	LP012
HBHM	Normal trachea	pSport1	LP012
HLFC	Human Larynx	pSport1	LP012
HLRB	Siebben Polyposis	pSport1	LP012
HNIA	Mammary Gland	pSport1	LP012
HNJB	Palate carcinoma	pSport1	LP012
HNKA	Palate normal	pSport1	LP012
HMZA	Pharynx carcinoma	pSport1	LP012
HABG	Cheek Carcinoma	pSport1	LP012
HMZM	Pharynx Carcinoma	pSport1	LP012
HDRM	Larynx Carcinoma	pSport1	LP012
HVAA	Pancreas normal PCA4 No	pSport1	LP012
HICA	Tongue carcinoma	pSport1	LP012
HUKA HUKB HUKC HUKD HUK E	Human Uterine Cancer	Lambda ZAP II	LP013
HFFA	Human Fetal Brain, random primed	Lambda ZAP II	LP013
HTUA	Activated T-cell labeled with 4-thioluri	Lambda ZAP II	LP013
HBQA	Early Stage Human Brain, random primed	Lambda ZAP II	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HMMA	Spleen metastatic melanoma	pSport 1	LP10
HLYA HLYB HLYC HLYD HLYE	Spleen. Chronic lymphocytic leukemia	pSport 1	LP10
HCGA	CD34+ cell. 1	pSport 1	LP10
HEOM HEON	Human Eosinophils	pSport 1	LP10
HTDA	Human Tonsil. Lib 3	pSport 1	LP10
HSPA	Salivary Gland, Lib 2	pSport 1	LP10
HCHA HCHB HCHC	Breast Cancer cell line, MDA 36	pSport 1	LP10
HCHM HCHN	Breast Cancer Cell line, angiogenic	pSport 1	LP10
HCIA	Crohn's Disease	pSport 1	LP10
HDAA HDAB HDAC	HEL cell line	pSport 1	LP10
HABA	Human Astrocyte	pSport 1	LP10
HUFA HUFB HUFC	Ulcerative Colitis	pSport 1	LP10
HNTM	NTERA2 + retinoic acid, 14 days	pSport 1	LP10
HDQA	Primary Dendritic cells, CapFinder2, frac 1	pSport 1	LP10
HDQM	Primary Dendritic Cells, CapFinder, frac 2	pSport 1	LP10
HLDX	Human Liver, normal, CapFinder	pSport 1	LP10
HULA HULB HULC	Human Dermal Endothelial Cells, untreated	pSport 1	LP10
HUMA	Human Dermal Endothelial cells, treated	pSport 1	LP10
HCJA	Human Stromal Endometrial fibroblasts, untreated	pSport 1	LP10
HCJM	Human Stromal endometrial fibroblasts, treated w/ estradiol	pSport 1	LP10
HEDA	Human Stromal endometrial fibroblasts, treated with progesterone	pSport 1	LP10
HFNA	Human ovary tumor cell OV350721	pSport 1	LP10
HKGA HKGB HKGC HKGD	Merkel Cells	pSport 1	LP10
HISA HISB HISC	Pancreas Islet Cell Tumor	pSport 1	LP10
HLSA	Skin, burned	pSport 1	LP10
HBZA	Prostate, BPH, Lib 2	pSport 1	LP10
HBZS	Prostate BPH, Lib 2, subtracted	pSport 1	LP10
HFIA HFIB HFIC	Synovial Fibroblasts (control)	pSport 1	LP10
HFIH HFII HFIJ	Synovial hypoxia	pSport 1	LP10
HFIT HFIU HFIV	Synovial IL-1/TNF stimulated	pSport 1	LP10
HGCA	Mesangial cell, frac 1	pSport 1	LP10
HMVA HMVB HMVC	Bone Marrow Stromal Cell, untreated	pSport 1	LP10
HFIX HFIZ HFIZ	Synovial Fibroblasts (IL1/TNF), sub	pSport 1	LP10
HFOX HFOY HFOZ	Synovial hypoxia-RSF subtracted	pSport 1	LP10
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP11
HLIA HLIB HLIC	Human Liver	pCMVSPORT 1	LP012
HHBA HHBB HHBC HHBD HHBE	Human Heart	pCMVSPORT 1	LP012
HBBA HBBB	Human Brain	pCMVSPORT 1	LP012
HLJA HLJB HLJC HLJD HLJE	Human Lung	pCMVSPORT 1	LP012
HOGA HOGB HOGC	Ovarian Tumor	pCMVSPORT 2.0	LP012



Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HLJA HLJB	Human Lung	pCMVSPORT 1	LP06
HOFM HOFN HOFO	H. Ovarian Tumor, II. OV5232	pCMVSPORT 2.0	LP07
HOGA HOGB HOGC	OV 10-3-95	pCMVSPORT 2.0	LP07
HCGL	CD34+cells, II	pCMVSPORT 2.0	LP07
HDLA	Hodgkin's Lymphoma I	pCMVSPORT 2.0	LP07
HDTA HDTB HDTC HDTD HDTE	Hodgkin's Lymphoma II	pCMVSPORT 2.0	LP07
HKAA HKAB HKAC HKAD HKAE HKAF HKAG HKAH	Keratinocyte	pCMVSPORT 2.0	LP07
HCIM	CAPFINDER, Crohn's Disease, lib 2	pCMVSPORT 2.0	LP07
HKAL	Keratinocyte, lib 2	pCMVSPORT 2.0	LP07
HKAT	Keratinocyte, lib 3	pCMVSPORT 2.0	LP07
HNDA	Nasal polyps	pCMVSPORT 2.0	LP07
HDRA	H. Primary Dendritic Cells, lib 3	pCMVSPORT 2.0	LP07
HOHA HOHB HOHC	Human Osteoblasts II	pCMVSPORT 2.0	LP07
HLDA HLDB HLDC	Liver, Hepatoma	pCMVSPORT 3.0	LP08
HLDN HLDO HLDP	Human Liver, normal	pCMVSPORT 3.0	LP08
HMTA	pBMC stimulated w/ poly I/C	pCMVSPORT 3.0	LP08
HNTA	NTERA2, control	pCMVSPORT 3.0	LP08
HDP A HDPB HDP C HDPD HDPF HDPG HDPH HDPI HDPJ HDPK	Primary Dendritic Cells, lib 1	pCMVSPORT 3.0	LP08
HDP M HDP N HDPO HDPP	Primary Dendritic cells, frac 2	pCMVSPORT 3.0	LP08
HMUA HMUB HMUC	Myeloid Progenitor Cell Line	pCMVSPORT 3.0	LP08
HHEA HHEB HHEC HHED	T Cell helper I	pCMVSPORT 3.0	LP08
HHEM HHEN HHEO HHEP	T cell helper II	pCMVSPORT 3.0	LP08
HEQA HEQB HEQC	Human endometrial stromal cells	pCMVSPORT 3.0	LP08
HJMA HJMB	Human endometrial stromal cells-treated with progesterone	pCMVSPORT 3.0	LP08
HSWA HSWB HSWC	Human endometrial stromal cells-treated with estradiol	pCMVSPORT 3.0	LP08
HSYA HSYB HSYC	Human Thymus Stromal Cells	pCMVSPORT 3.0	LP08
HLWA HLWB HLWC	Human Placenta	pCMVSPORT 3.0	LP08
HRAA HRAB HRAC	Rejected Kidney, lib 4	pCMVSPORT 3.0	LP08
HMTM	PCR, pBMC I/C treated	PCR II	LP09
HMJA	H. Meningioma, M6	pSport 1	LP10
HMKA HMKB HMKC HMKD HMKE	H. Meningioma, M1	pSport 1	LP10
HUSG HUSI	Human umbilical vein endothelial cells, IL-4 induced	pSport 1	LP10
HUSX HUSY	Human Umbilical Vein Endothelial Cells, uninduced	pSport 1	LP10
HOFA	Ovarian Tumor I, OV5232	pSport 1	LP10
HCFA HCFB HCFC HCFC	T-Cell PHA 16 hrs	pSport 1	LP10
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport 1	LP10
HADA HADC HADD HADE HADF HADG	Human Adipose	pSport 1	LP10
HOVA HOVB HOVC	Human Ovary	pSport 1	LP10
HTWB HTWC HTWD HTWE HTWF	Resting T-Cell Library, II	pSport 1	LP10

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP04
HELA HELB HELC HELD HELE HELF HELG HELH	Endothelial cells-control	Uni-ZAP XR	LP04
HEMA HEMB HEMC HEMD HEME HEMF HEMG HEMH	Endothelial-induced	Uni-ZAP XR	LP04
HBIA HBIB HBIC	Human Brain, Striatum	Uni-ZAP XR	LP04
HHS A HHSB HHSC HHSD HHSE	Human Hypothalamus.Schizophrenia	Uni-ZAP XR	LP04
HNGA HNGB HNGC HNGD HNGE HNGF HNGG HNGH HNGI HNGJ	neutrophils control	Uni-ZAP XR	LP04
HNHA HNHB HNHC HNHD HNHE HNHF HNHG HNHH HNHI HNHI HSDB HSDC	Neutrophils IL-1 and LPS induced	Uni-ZAP XR	LP04
HHPT	STRIATUM DEPRESSION	Uni-ZAP XR	LP04
HSAT HSAU HSAV HSAW HSAX HSAY HSAZ	Hypothalamus	Uni-ZAP XR	LP04
HBMS HBMT HBMU HBMV HBMW HBMX	Anergic T-cell	Uni-ZAP XR	LP04
HOEA HOEB HOEC HOED HOEE HOEF HOEJ	Bone marrow	Uni-ZAP XR	LP04
HAIA HAIB HAIC HAID HAIE HAIF	Osteoblasts	Uni-ZAP XR	LP04
HTGA HTGB HTGC HTGD	Epithelial-TNF $\alpha$ and INF induced	Uni-ZAP XR	LP04
HMCA HMCB HMCC HMCD HMCE	Apoptotic T-cell	Uni-ZAP XR	LP04
HMAA HMAB HMA C HMAD HMAE HMAF HMAG	Macrophage-oxLDL	Uni-ZAP XR	LP04
HPHA	Macrophage (GM-CSF treated)	Uni-ZAP XR	LP04
HPIA HPIB HPIC	Normal Prostate	Uni-ZAP XR	LP04
HPJA HPJB HPJC	LNCAP prostate cell line	Uni-ZAP XR	LP04
HOSE HOSF HOSG	PC3 Prostate cell line	Uni-ZAP XR	LP04
HTGE HTGF	Human Osteoclastoma, re-excision	Uni-ZAP XR	LP04
HMAJ HMAK	Apoptotic T-cell, re-excision	Uni-ZAP XR	LP04
HACB HACC HACD	H Macrophage (GM-CSF treated), re-excision	Uni-ZAP XR	LP04
HFAA HFAB HFAC HFAD HFAE	Human Adipose Tissue, re-excision	Uni-ZAP XR	LP04
HFAM	H. Frontal Cortex, Epileptic	Uni-ZAP XR	LP04
HMIA HMIB HMIC	Alzheimers, spongy change	Uni-ZAP XR	LP04
HTSA HTSE HTSF HTSG HTSH	Frontal Lobe, Dementia	Uni-ZAP XR	LP04
HPBA HPBB HPBC HPBD HPBE	Human Manic Depression Tissue	Uni-ZAP XR	LP04
HSAA HSAB HSAC	Human Thymus	pBS	LP05
HSBA HSBB HSBC HSBM	Human Pineal Gland	pBS	LP05
HJAA HJAB HJAC HJAD	HSA 172 Cells	pBS	LP05
HJBA HJBB HJBC HJBD	HSC172 cells	pBS	LP05
HFAA HAFB	Jurkat T-cell G1 phase	pBS	LP05
HAWA HAWB HAWC	Jurkat T-Cell, S phase	pBS	LP05
HTNA HTNB	Aorta endothelial cells + TNF- $\alpha$	pBS	LP05
HONA	Human White Adipose	pBS	LP05
HARA HARB	Human Thyroid	pBS	LP05
	Normal Ovary, Premenopausal	pBS	LP05
	Human Adult Retina	pBS	LP05

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
	excision		
HMSA HMSB HMSC HMSD HMSE HMSF HMSG HMSH HMSI HMSJ HMSK	Monocyte activated	Uni-ZAP XR	LP03
HAGA HAGB HAGC HAGD HAGE HAGF	Human Amygdala	Uni-ZAP XR	LP03
HSRA HSRB HSRE	STROMAL -OSTEOCLASTOMA	Uni-ZAP XR	LP03
HSRD HSRF HSRG HSRH	Human Osteoclastoma Stromal Cells - unamplified	Uni-ZAP XR	LP03
HSQA HSQB HSQC HSQD HSQE HSQF HSQG	Stromal cell TF274	Uni-ZAP XR	LP03
HSKA HSKB HSKC HSKD HSKE HSKF HSKZ	Smooth muscle, serum treated	Uni-ZAP XR	LP03
HSLA HSLB HSLC HSLD HSLF HSLG	Smooth muscle, control	Uni-ZAP XR	LP03
HSDA HSDD HSDE HSDF HSDG HSDH	Spinal cord	Uni-ZAP XR	LP03
HPWS	Prostate-BPH subtracted II	pBS	LP03
HSKW HSKX HSKY	Smooth Muscle- HASTE normalized	pBS	LP03
HFPB HFPC HFPD	H. Frontal cortex, epileptic; re-excision	Uni-ZAP XR	LP03
HSDI HSDJ HSDK	Spinal Cord, re-excision	Uni-ZAP XR	LP03
HSKN HSKO	Smooth Muscle Serum Treated, Norm	pBS	LP03
HSKG HSKH HSKI	Smooth muscle, serum induced, re-exc	pBS	LP03
HFCA HFCB HFCC HFCD HFCE HFCF	Human Fetal Brain	Uni-ZAP XR	LP04
HPTA HPTB HPTD	Human Pituitary	Uni-ZAP XR	LP04
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP04
HE6B HE6C HE6D HE6E HE6F HE6G HE6S	Human Whole Six Week Old Embryo	Uni-ZAP XR	LP04
HSSA HSSB HSSC HSSD HSSE HSSF HSSG HSSH HSSI HSSJ HSSK HE7T	Human Synovial Sarcoma	Uni-ZAP XR	LP04
	7 Week Old Early Stage Human, subtracted	Uni-ZAP XR	LP04
HEPA HEPB HEPC	Human Epididymus	Uni-ZAP XR	LP04
HSNA HSNB HSNB HSNM HSNM	Human Synovium	Uni-ZAP XR	LP04
HPFB HPFC HPFD HPFE	Human Prostate Cancer, Stage C fraction	Uni-ZAP XR	LP04
HE2A HE2D HE2E HE2H HE2I HE2M HE2N HE2O	12 Week Old Early Stage Human	Uni-ZAP XR	LP04
HE2B HE2C HE2F HE2G HE2P HE2Q	12 Week Old Early Stage Human, II	Uni-ZAP XR	LP04
HPTS HPTT HPTU	Human Pituitary, subtracted	Uni-ZAP XR	LP04
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP04
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP04
HWTA HWTB HWTG	wilm's tumor	Uni-ZAP XR	LP04
HBSD	Bone Cancer, re-excision	Uni-ZAP XR	LP04
HSGB	Salivary gland, re-excision	Uni-ZAP XR	LP04
HSJA HSJB HSJC	Smooth muscle-IL1b induced	Uni-ZAP XR	LP04
HSXA HSXB HSXC HSXD	Human Substantia Nigra	Uni-ZAP XR	LP04
HSJA HSJB HSJC	Smooth muscle, IL1b induced	Uni-ZAP XR	LP04
HOUA HOUB HOUC HOUD HOUE	Adipocytes	Uni-ZAP XR	LP04

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HSUT	Supt cells, cyclohexamide treated, differentially expressed	pBS	LP03
HT4S	Activated T-Cells, 12 hrs. subtracted	Uni-ZAP XR	LP03
HCDA HCDB HCDC HCDD HCDE	Human Chondrosarcoma	Uni-ZAP XR	LP03
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP03
HTLA HTLB HTLC HTLD HTLE HTLF	Human adult testis, large inserts	Uni-ZAP XR	LP03
HLMA HLMC HLMD	Breast Lymph node cDNA library	Uni-ZAP XR	LP03
H6EA H6EB H6EC	HL-60, PMA 4H	Uni-ZAP XR	LP03
HTXA HTXB HTXC HTXD HTXE HTXF HTXG HTXH	Activated T-Cell (12hs)/Thiouridine labelled Eco	Uni-ZAP XR	LP03
HNFA HNFB HNFC HNFD HNFE HNFF HNFG HNFH HNFJ	Human Neutrophil, Activated	Uni-ZAP XR	LP03
HTOB HTOC	HUMAN TONSILS, FRACTION 2	Uni-ZAP XR	LP03
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP03
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP03
HORB	Human OB HOS treated (10 nM E2) fraction I	Uni-ZAP XR	LP03
HSVA HSVB HSVC	Human Chronic Synovitis	Uni-ZAP XR	LP03
HROA	HUMAN STOMACH	Uni-ZAP XR	LP03
HBJA HBJB HBJC HBJD HBJE HBJF HBJG HBJH HBJI HBJJ HBJK	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP03
HCRA HCRB HCRC	human corpus colosum	Uni-ZAP XR	LP03
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP03
HDSA	Dermatofibrosarcoma Protuberance	Uni-ZAP XR	LP03
HMWA HMWB HMWC HMWD HMWE HMWF HMWG HMWH HMWI HMWJ	Bone Marrow Cell Line (RS4;11)	Uni-ZAP XR	LP03
HSOA	stomach cancer (human)	Uni-ZAP XR	LP03
HERA	SKIN	Uni-ZAP XR	LP03
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP03
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP03
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP03
HBCA HBCB	H. Lymph node breast Cancer	Uni-ZAP XR	LP03
HPWT	Human Prostate BPH, re-excision	Uni-ZAP XR	LP03
HFVG HFVH HFVI	Fetal Liver, subtraction II	pBS	LP03
HNFI	Human Neutrophils, Activated, re-excision	pBS	LP03
HBMB HBMC HBMD	Human Bone Marrow, re-excision	pBS	LP03
HKML HKMM HKMN	H. Kidney Medulla, re-excision	pBS	LP03
HKIX HKIY	H. Kidney Cortex, subtracted	pBS	LP03
HADT	H. Amygdala Depression, subtracted	pBS	LP03
H6AS	HL-60, untreated, subtracted	Uni-ZAP XR	LP03
H6ES	HL-60, PMA 4H, subtracted	Uni-ZAP XR	LP03
H6BS	HL-60, RA 4h, Subtracted	Uni-ZAP XR	LP03
H6CS	HL-60, PMA 1d, subtracted	Uni-ZAP XR	LP03
HTXJ HTXK	Activated T-cell(12h)/Thiouridine-re-	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HLHF HLHG HLHH HLHQ			
HPMA HPMB HPMC HPMD HPME HPMF HPMG HPMH	Human Placenta	Uni-ZAP XR	LP03
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP03
HSIA HSIC HSID HSIE	Human Adult Small Intestine	Uni-ZAP XR	LP03
HTEA HTEB HTEC HTEH HTEI HTEJ HTEK	Human Testes	Uni-ZAP XR	LP03
HTPA HTPB HTPC HTPD HTPF	Human Pancreas Tumor	Uni-ZAP XR	LP03
HTTA HTTB HTTC HTTD HTTE HTTF	Human Testes Tumor	Uni-ZAP XR	LP03
HAPA HAPB HAPC HAPM	Human Adult Pulmonary	Uni-ZAP XR	LP03
HETA HETB HETC HETD HETE HETF HETG HETH HETI	Human Endometrial Tumor	Uni-ZAP XR	LP03
HHFB HHFC HHFD HHFE HHFF HHFG HHFH HHFI	Human Fetal Heart	Uni-ZAP XR	LP03
HHPB HHPC HHPD HHPE HHPF HHPG HHPH	Human Hippocampus	Uni-ZAP XR	LP03
HCE1 HCE2 HCE3 HCE4 HCE5 HCEB HCEC HCED HCEE HCEF HCEG	Human Cerebellum	Uni-ZAP XR	LP03
HUVB HUVC HUVD HUVE	Human Umbilical Vein, Endo. remake	Uni-ZAP XR	LP03
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP03
HTAA HTAB HTAC HTAD HTAE	Human Activated T-Cells	Uni-ZAP XR	LP03
HFEA HFEB HFEC	Human Fetal Epithelium (Skin)	Uni-ZAP XR	LP03
HJPA HJPB HJPC HJPD	HUMAN JURKAT MEMBRANE BOUND POLYSOMES	Uni-ZAP XR	LP03
HESA	Human epithelioid sarcoma	Uni-Zap XR	LP03
HLTA HLTB HLTC HLTD HLTE HLTF	Human T-Cell Lymphoma	Uni-ZAP XR	LP03
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP03
HRDA HRDB HRDC HRDD HRDE HRDF	Human Rhabdomyosarcoma	Uni-ZAP XR	LP03
HCAA HCAB HCAC	Cem cells cyclohexamide treated	Uni-ZAP XR	LP03
HRGA HRGB HRGC HRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP03
HSUA HSUB HSUC HSUM	Supt Cells, cyclohexamide treated	Uni-ZAP XR	LP03
HT4A HT4C HT4D	Activated T-Cells, 12 hrs.	Uni-ZAP XR	LP03
HE9A HE9B HE9C HE9D HE9E HE9F HE9G HE9H HE9M HE9N	Nine Week Old Early Stage Human	Uni-ZAP XR	LP03
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP03
HT5A	Activated T-Cells, 24 hrs.	Uni-ZAP XR	LP03
HFGA HFGM	Human Fetal Brain	Uni-ZAP XR	LP03
HNEA HNEB HNEC HNED HNEE	Human Neutrophil	Uni-ZAP XR	LP03
HBGB HBGD	Human Primary Breast Cancer	Uni-ZAP XR	LP03
HBNA HBNB	Human Normal Breast	Uni-ZAP XR	LP03
HCAS	Cem Cells, cyclohexamide treated, subtra	Uni-ZAP XR	LP03
HHPS	Human Hippocampus, subtracted	pBS	LP03
HKCS HKCU	Human Colon Cancer, subtracted	pBS	LP03
HRGS	Raji cells, cyclohexamide treated, subtracted	pBS	LP03

TABLE 5

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HUKA HUKB HUKC HUKD HUKF HUKG	Human Uterine Cancer	Lambda ZAP II	LP01
HCNA HCNB	Human Colon	Lambda Zap II	LP01
HFFA	Human Fetal Brain, random primed	Lambda Zap II	LP01
HTWA	Resting T-Cell	Lambda ZAP II	LP01
HBQA	Early Stage Human Brain, random primed	Lambda ZAP II	LP01
HLMB HLMF HLMG HLMH HLMI HLMJ HLMM HLMN	breast lymph node CDNA library	Lambda ZAP II	LP01
HCQA HCQB	human colon cancer	Lambda ZAP II	LP01
HMEA HMEC HMED HMEF HMEG HMEI HMEJ HMEK HMEI	Human Microvascular Endothelial Cells, fract. A	Lambda ZAP II	LP01
HUSA HUSC	Human Umbilical Vein Endothelial Cells, fract. A	Lambda ZAP II	LP01
HLQA HLQB	Hepatocellular Tumor	Lambda ZAP II	LP01
HHGA HHGB HHGC HHGD	Hemangiopericytoma	Lambda ZAP II	LP01
HSDM	Human Striatum Depression, re-rescue	Lambda ZAP II	LP01
HUSH	H Umbilical Vein Endothelial Cells, fract. A, re-excision	Lambda ZAP II	LP01
HSGS	Salivary gland, subtracted	Lambda ZAP II	LP01
HFXA HFXB HFXC HFXD HFXE HFXF HFXG HFXH	Brain frontal cortex	Lambda ZAP II	LP01
HPQA HPQB HPQC	PERM TF274	Lambda ZAP II	LP01
HFXJ HFXK	Brain Frontal Cortex, re-excision	Lambda ZAP II	LP01
HCWA HCWB HCWC HCWD HCWE HCWF HCWG HCWH HCWI HCWJ HCWK	CD34 positive cells (Cord Blood)	ZAP Express	LP02
HCUA HCUB HCUC	CD34 depleted Buffy Coat (Cord Blood)	ZAP Express	LP02
HRSM	A-14 cell line	ZAP Express	LP02
HRSA	A1-CELL LINE	ZAP Express	LP02
HCUD HCUE HCUF HCUG HCUH HCUI	CD34 depleted Buffy Coat (Cord Blood), re-excision	ZAP Express	LP02
HBXE HBXF HBXG	H. Whole Brain #2, re-excision	ZAP Express	LP02
HRLM	L8 cell line	ZAP Express	LP02
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP02
HUDA HUDB HUDC	Testes	ZAP Express	LP02
HHTM HHTN HHTO	H. hypothalamus, frac A; re-excision	ZAP Express	LP02
HHTL	H. hypothalamus, frac A	ZAP Express	LP02
HASA HASD	Human Adult Spleen	Uni-ZAP XR	LP03
HFKC HFKD HFKE HFKF HFKG	Human Fetal Kidney	Uni-ZAP XR	LP03
HE8A HE8B HE8C HE8D HE8E HE8F HE8M HE8N	Human 8 Week Whole Embryo	Uni-ZAP XR	LP03
HGBA HGBD HGBE HGBF HGBG HGBH HGBI	Human Gall Bladder	Uni-ZAP XR	LP03
HLHA HLHB HLHC HLHD HLHE	Human Fetal Lung III	Uni-ZAP XR	LP03

primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 5, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited by reference to Table 2 and 5 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone referenced in Table 1.

*Examples**Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample*

5 Each deposited cDNA clone is contained in a plasmid vector. Table 5 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The following correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a  
 10 particular clone is identified in Table 5 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited Plasmid</u>
	Lambda Zap	pBluescript (pBS)
	Uni-Zap XR	pBluescript (pBS)
15	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
	pCMVSPORT 3.0	pCMVSPORT 3.0
20	pCR <sup>®</sup> 2.1	pCR <sup>®</sup> 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 25 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3  
 30



an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to increase the level of said protein activity in said individual.

- 5        Also preferred is a method of treatment of an individual in need of a decreased level of a protein activity, which method comprised administering to such an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to decrease the level of  
10       said protein activity in said individual.

      Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence  
5 selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said  
10 polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

15 Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

20 Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a human protein comprising an amino acid sequence selected from  
25 the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an  
30 increased level of a protein activity, which method comprises administering to such

and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleic acid sequence identified in Table 1 encoding a polypeptide, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

5        Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a  
10       sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is a method for detecting in a biological sample a  
15       polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1; which method comprises a step of comparing an amino acid  
20       sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino  
25       acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a  
30       polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X;

polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
5 at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated polypeptide comprising an amino acid  
10 sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated polypeptide comprising an amino acid  
15 sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated polypeptide comprising an amino acid  
20 sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino  
acids is included in the amino acid sequence of a portion of said polypeptide encoded by the cDNA clone referenced in Table 1; a polypeptide encoded by SEQ ID NO:X;  
25 and/or the polypeptide sequence of SEQ ID NO:Y.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide  
5 sequence of the cDNA in the related cDNA clone contained in the deposit.

Also preferred is the above method for diagnosing a pathological condition which comprises a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous  
10 nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a  
15 sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is a composition of matter comprising isolated nucleic acid  
20 molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a DNA microarray or "chip" of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 100, 150, 200, 250, 300, 500, 1000, 2000, 3000 or 4000 nucleotide sequences, wherein at least one sequence in said DNA microarray or "chip" is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected  
25 from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the cDNA clone referenced in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
30 at least 90% identical to a sequence of at least about 10 contiguous amino acids in the

complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample which comprises a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleotide sequence of SEQ ID NO:X; or the cDNA in the related cDNA clone identified in Table 1 which encodes a protein, wherein the method comprises a step of detecting in a biological sample

the related cDNA clone contained in the deposit, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

5        Also preferred is a composition of matter comprising a DNA molecule which comprises a cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of the cDNA in the related cDNA clone  
10       contained in the deposit.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of an open reading frame sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

15       Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is an isolated nucleic acid molecule  
20       comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete  
25       nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence  
30       selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the



Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions identified as "Start" and "End" in columns 7 and 8 as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X in the range of positions identified as "Start" and "End" in columns 7 and 8 as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

5 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of  
10 energy.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or  
15 Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals,  
20 cofactors or other nutritional components.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit,  
25 goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

#### Other Preferred Embodiments

the present invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for treating wounds due to injuries, burns, post-operative tissue  
5 repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed stimulate neuronal growth and to treat and prevent neuronal  
10 damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone  
15 grafts.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention  
20 may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

25 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic  
5 cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular  
10 cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

15 The antagonist/agonist may also be employed to treat the diseases described herein.

Thus, the invention provides a method of treating disorders or diseases, including but not limited to the disorders or diseases listed throughout this application, associated with overexpression of a polynucleotide of the present  
20 invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

#### Other Activities

25 A polypeptide, polynucleotide, agonist, or antagonist of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The polypeptide, polynucleotide, agonist, or antagonist of

commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to the coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of SEQ ID NO:X. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy

5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 5 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, 10 queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 15 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a 20 phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands 25 run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Polynucleotides of the invention may be synthesized by standard methods 30 known in the art, e.g. by use of an automated DNA synthesizer (such as are

3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of polynucleotide sequences described herein could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of mRNA of the present invention, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre et al., 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil,

invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors  
5 can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding the polypeptide of the present invention or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter  
10 region (Bernoist and Chambon, Nature 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., Nature 296:39-42 (1982)), etc.

15 The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of the present invention. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the  
20 RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a  
25 stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the  
30 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the



Neurochem. 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl<sub>2</sub>, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the

complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability  
5 of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein.  
10 Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned  
15 drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides  
20 or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

#### **Antisense And Ribozyme (Antagonists)**

25 In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained in the cDNA contained in the related cDNA clone identified in Table 1. In one embodiment, antisense sequence is generated internally, by the organism, in another embodiment,  
30 the antisense sequence is separately administered (see, for example, O'Connor, J.,

toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

#### 10 Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a

activity, and (b) determining if a biological activity of the polypeptide has been altered.

### **Targeted Delivery**

5           In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

          As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or  
10       prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic  
15       protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

          In another embodiment, the invention provides a method for the specific  
20       destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

          By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of  
25       toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced  
30       endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha

thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of  $^3\text{[H]}$  thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of  $^3\text{[H]}$  thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological

33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and  
5 corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior  
10 to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments,  
15 the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation  
20 factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present  
25 invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the  
30 polypeptide of the present invention, the compound to be screened and  $^3\text{H}$

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed  
5 wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are  
10 exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and  
15 re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and  
20 exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

25 Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721,  
30 5,834,252, and 5,837,458, and Patten, P. A., et al., *Curr. Opinion Biotechnol.* 8:724-

(antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.



(e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

5

### Chemotaxis

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

### Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit

### Regeneration

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of  
5 tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs  
10 (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists  
15 of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome,  
20 and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using  
polynucleotides or polypeptides, as well as agonists or antagonists of the present  
25 invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or  
30 other medical therapies), localized neuropathies, and central nervous system diseases

Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, Polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, Diphtheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more  
5 other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or  
10 antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., *Corynebacterium*, *Mycobacterium*, *Nocardia*), *Cryptococcus neoformans*, *Aspergillus*, *Bacillaceae* (e.g., *Anthrax*, *Clostridium*), *Bacteroidaceae*, *Blastomycosis*, *Bordetella*, *Borrelia* (e.g., *Borrelia burgdorferi*,  
15 *Brucellosis*, *Candidiasis*, *Campylobacter*, *Coccidioidomycosis*, *Cryptococcosis*, *Dermatocycoses*, *E. coli* (e.g., *Enterotoxigenic E. coli* and *Enterohemorrhagic E. coli*), *Enterobacteriaceae* (*Klebsiella*, *Salmonella* (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), *Serratia*, *Yersinia*), *Erysipelothrix*, *Helicobacter*, *Legionellosis*, *Leptospirosis*, *Listeria*, *Mycoplasmatales*, *Mycobacterium leprae*,  
20 *Vibrio cholerae*, *Neisseriaceae* (e.g., *Acinetobacter*, *Gonorrhea*, *Menigococcal*), *Meisseria meningitidis*, *Pasteurellacea Infections* (e.g., *Actinobacillus*, *Heamophilus* (e.g., *Heamophilus influenza type B*), *Pasteurella*), *Pseudomonas*, *Rickettsiaceae*, *Chlamydiaceae*, *Syphilis*, *Shigella spp.*, *Staphylococcal*, *Meningiococcal*, *Pneumococcal* and *Streptococcal* (e.g., *Streptococcus pneumoniae* and Group B  
25 *Streptococcus*). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme  
30 Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning,

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by  
5 initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or  
10 symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae,  
15 Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g.,  
20 Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis,  
25 Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention,  
30 can be used to treat or detect any of these symptoms or diseases. In specific

- and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis, Multiplex Paramyoclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia,
- 5 amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia, Melkersson-
- 10 Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis, Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic
- 15 Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as Neuromyelitis Optica and Swayback, Diabetic neuropathies such as diabetic foot, nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve
- 20 compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia
- 25 and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

### Infectious Disease

meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta, hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such

5 as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia,

10 broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium,

15 fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as

20 diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision

25 defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron

30 disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease

Pneumococcal Meningitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uvemeningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and

5 postpoliomyelitis syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie) cerebral toxoplasmosis, central nervous system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and

10 supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral scleritis which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic

15 encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord

20 compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon- Biedl Syndrome, Lesch-Nyhan

25 Syndrome, Maple Syrup Urine Disease, mucopolysaccharidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes

30 hydranencephaly, Arnold-Chiari Deformity, encephalocele, meningocele,



- thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency,
- 5   vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache, migraine, dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis
- 10   which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular
- 15   leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, Hallervorden-Spatz Syndrome, hydrocephalus such as
- 20   Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia
- 25   Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, cerebral malaria, meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis which includes lymphocytic choriomeningitis. Bacterial meningitis which includes Haemophilus Meningitis, Listeria Meningitis,
- 30   Meningococcal Meningitis such as Waterhouse-Friderichsen Syndrome,

polypeptides, as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

## 5 Neurological Diseases

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to  
10 stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

15 Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain  
20 edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph  
25 Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis, cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease, cerebral  
30 amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and

production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associate with the under expression.

5           Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent  
10   or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as  
15   agonists or antagonists of the present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

          Polynucleotides or polypeptides, as well as agonists or antagonists of the  
20   present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

25           In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to maintain the islet function so as to alleviate,  
30   delay or prevent permanent manifestation of the disease. Also, polynucleotides or

epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the

healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avascular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentum graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of

lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

25

#### **Wound Healing and Epithelial Cell Proliferation**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound

30

### Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma,

molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxyaminolmidazole; and metalloproteinase inhibitors such as BB94.



preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide  
5 variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present  
10 invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d  
15 group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

20 Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate  
25 including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten  
30 oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo

peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch  
5 granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal  
10 surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic  
15 compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-  
20 angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the  
25 site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly

compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, 5 granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, 10 Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, 15 retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, 20 hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary 25 angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or 30 agonists may also be used in controlling menstruation or administered as either a

after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The

capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

10        Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, 15        prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in 20        corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

25        Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the 30        cornea from the advancing blood vessels. This method may also be utilized shortly

telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a  
5 polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of  
10 particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases  
15 of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma,  
20 diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalm.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalm.* 22:291-312 (1978).

25 Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue  
30 which normally lacks blood vessels. In certain pathological conditions however,

may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including lung, prostate, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, 5 parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, 10 in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists 15 may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in 20 treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artherosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular 25 glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; 30 ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization;

including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun,  
10 *Science* 235:442-447 (1987).

The polynucleotides encoding a polypeptide of the present invention may be administered along with other polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha  
15 and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

The present invention provides for treatment of diseases or disorders  
20 associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and  
25 otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the  
30 invention. For example, polynucleotides, polypeptides, antagonists and/or agonists



Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome,  
5 thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

10 Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or  
15 topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

#### 20 Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound  
25 healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases.  
30 A number of serious diseases are dominated by abnormal neovascularization

Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease,  
5 ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus  
10 syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia,  
15 Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve  
20 insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial  
25 reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms,  
30 angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease,

polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic  
5 and/or covalent interactions. Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the  
10 expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

#### **Cardiovascular Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the present  
15 invention, may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects  
20 include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion  
25 defects, Lutembacher's Syndrome, trilogly of Fallot, ventricular heart septal defects.

Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart  
30 hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right

Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous

the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering  
5 a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or  
10 hematopoietic growth factors, for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and  
15 therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  
20  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere  
25 herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may  
30 also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al.,

polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the  
5 destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in  
10 the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature  
15 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating  
20 and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for  
25 polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The



hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

- 5           One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

          Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

- 10           Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

5

#### Hyperproliferative Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by Polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to:

Examples of autoimmune disorders that can be treated or detected include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

10 Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

15 Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells  
20 destroy the host tissues. The administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides or polypeptides, or agonists or antagonists of the  
25 present invention may also be used to modulate inflammation. For example, polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and  
30 malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or

hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV  
5 infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

10 Moreover, polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat blood coagulation disorders  
15 (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of  
20 heart attacks (infarction), strokes, or scarring.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response  
25 leading to the destruction of the host tissue. Therefore, the administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

5

### **Biological Activities**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention,  
10 do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides, and agonists or antagonists could be used to treat the associated disease.

### 15 **Immune Activity**

A polypeptide or polynucleotide, or agonists or antagonists of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called  
20 hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides or polypeptides, or agonists or antagonists  
25 of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. Polynucleotides or polypeptides, or agonists or antagonists of the  
30 present invention could be used to increase differentiation and proliferation of

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue  
5 inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a  
10 particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad.  
15 Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a  
20 polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise  
25 condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

Preferably, the polynucleotide encoding a polypeptide of the present invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

polynucleotide construct integrated into its genome, and will express a polypeptide of the invention.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can



products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or  
5 packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

10 In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300  
15 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will  
20 include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper  
25 virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce  
30 eukaryotic cells, either ex vivo or in vivo. The transduced cells will contain the

the use of liposomes, and  $\text{CaPO}_4$  precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

5 The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding a polypeptide of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express a polypeptide of the present invention.

10 In certain other embodiments, cells are engineered, ex vivo or in vivo, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses a polypeptide of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis.

15 Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis. 109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish

20 adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993);

25 Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively

30 express Ela and Elb, which complement the defective adenoviruses by providing the

Sci. USA (1979) 76:145); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem. (1980) 255:10431; Szoka, F. and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA (1978) 75:145; Schaefer-Ridder et al., Science (1982) 215:166), which are herein incorporated by reference.

5           Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

          U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into  
10   mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are  
15   herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

          In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding a polypeptide of the present invention. Retroviruses from which the retroviral plasmid  
20   vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

          The retroviral plasmid vector is employed to transduce packaging cell lines to  
25   form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any  
30   means known in the art. Such means include, but are not limited to, electroporation,

be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

10       The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology* (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77); ether injection (Deamer, D. and Bangham, A., *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al., *Proc. Natl. Acad. Sci. USA* (1979) 76:3348); detergent dialysis (Enoch, H. and Strittmatter, P., *Proc. Natl. Acad.*

herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

5           Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially  
10       available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of  
15       DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from  
20       Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP  
25       starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with  
30       or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can

cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is

Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotide of the present invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated

(1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotide of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.



Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described supra, and elsewhere herein). For example, administration of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

#### 15 Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the present invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide of the present invention. Such methods are well-known in the art. For example, see Beldegrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a lung cancer polypeptide of the present invention in cells or body fluid of an individual, or more preferably, assaying the expression level of a lung cancer polypeptide of the present invention in lung cells or sputum of an individual; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, lung cancer antigen polypeptides of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions, preferably proliferative disorders of the lung, and/or cancerous disease and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

- 5 In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or  
10 on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or  
15 induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, <sup>213</sup>Bi, or other  
20 radioisotopes such as, for example, <sup>103</sup>Pd, <sup>133</sup>Xe, <sup>131</sup>I, <sup>68</sup>Ge, <sup>57</sup>Co, <sup>65</sup>Zn, <sup>85</sup>Sr, <sup>32</sup>P, <sup>35</sup>S, <sup>90</sup>Y, <sup>153</sup>Sm, <sup>153</sup>Gd, <sup>169</sup>Yb, <sup>51</sup>Cr, <sup>54</sup>Mn, <sup>75</sup>Se, <sup>113</sup>Sn, <sup>90</sup>Yttrium, <sup>117</sup>Tin, <sup>186</sup>Rhenium, <sup>166</sup>Holmium, and <sup>188</sup>Rhenium; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

- Techniques known in the art may be applied to label polypeptides of the  
25 invention (including antibodies). Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable  
5 characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ , ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium  
10 ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ,  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or  
15 intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled  
20 antibody or antibody fragment will then preferentially accumulate at the location of cells which express the polypeptide encoded by a polynucleotide of the invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson  
25 Publishing Inc. (1982)).

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., polypeptides encoded by polynucleotides of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In  
30 one example, the invention provides a method for delivering a therapeutic protein into

specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an  
5 immune response.

### Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

10 Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

15 Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression  
20 include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ),  
25 palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying levels of polypeptide of the present invention in a  
30 biological sample, proteins can also be detected in vivo by imaging. Antibody labels

Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with  
5 tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to lung or lung cancer polynucleotides prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

10 The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays) or cell type(s) (e.g.,  
15 immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention, lung and lung cancer tissues and/or cancerous and/or wounded tissues) or  
20 bodily fluids (e.g., sputum, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Thus, the invention provides a diagnostic method of a disorder, which  
25 involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby an increase or decrease in the assayed gene expression level compared to the standard expression level is indicative of a disorder.

In the very least, the polynucleotides of the present invention can be used as  
30 molecular weight markers on Southern gels, as diagnostic probes for the presence of a

manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene.

Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness is not limited to treatment of proliferative disorders of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

5           In addition to the foregoing, a lung cancer antigen polynucleotide can be used to control gene expression through triple helix formation or through antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for  
10 instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription  
15 (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while  
20 antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of polypeptide of the present invention antigens. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple  
25 helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the  
30 present invention offer a means of targeting such genetic defects in a highly accurate



pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans:

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in *Neoplastic Diseases of the Blood*, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., *supra*) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., *supra*) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., *supra*)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580). However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., *Proc. Natl. Acad. Sci.* 85:1028 (1988); Anfossi et al., *Proc. Natl.*

disorders, and/or cancerous diseases and conditions, though most preferably in lung related proliferative, and/or cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

5       The present invention encompasses lung cancer polynucleotides that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a  
10 polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt,  
15 L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide  
20 backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer  
25 lowers the melting point ( $T_{sub.m}$ ) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

30       The present invention have uses which include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of

cancer polypeptide or the level of the mRNA encoding the lung cancer polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the lung cancer polypeptide level or mRNA level in a second biological sample). Preferably, the lung cancer polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard lung cancer polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the lung related disorder or being determined by averaging levels from a population of individuals not having a lung related disorder. As will be appreciated in the art, once a standard lung cancer polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains lung cancer polypeptide or the corresponding mRNA. As indicated, biological samples include body fluids (such as sputum, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the lung cancer polypeptide, lung tissue, and other tissue sources found to express the lung cancer polypeptide. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with lung cancer polynucleotides attached may be used to identify polymorphisms between the lung cancer polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative

chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention provides a method of detecting increased or decreased expression levels of the lung cancer polynucleotides in affected individuals as compared to unaffected individuals using polynucleotides of the present invention and techniques known in the art, including but not limited to the method described in Example 11. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a lung related disorder, including lung cancer, involving measuring the expression level of lung cancer polynucleotides in lung tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard lung cancer polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a lung related disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the invention, where each probe has one strand containing a 31'mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a lung related disorder, including, for example, diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed lung cancer polynucleotide expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of lung cancer polynucleotides" is intended qualitatively or quantitatively measuring or estimating the level of the lung

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g. Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London  
5 (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location,  
10 the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).) Assuming 1 megabase mapping resolution and  
15 one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in a polynucleotide of the invention and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as  
20 deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required  
25 to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using the polynucleotides of the invention. Any of these alterations (altered expression,

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably at least 15 bp (e.g., 15-25 bp) from the sequences shown in SEQ ID NO:X, or the complement thereto. Primers can optionally be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids  
5 containing individual human chromosomes. Only those hybrids containing the human gene corresponding to SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned  
10 per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques  
15 (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however,  
20 polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for  
25 marking multiple sites and/or multiple chromosomes).

Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from the polynucleotide sequences in Table 3 and SEQ ID NO:X and (b) screening somatic cell hybrids containing individual chromosomes.

bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

#### Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The lung cancer antigen polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome, thus each polynucleotide of the present invention can routinely be used as a chromosome marker using techniques known in the art.

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of



Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

### ***Kits***

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that  
5 detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

10 It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99m</sup>Tc. The labeled antibody or antibody fragment will then preferentially  
15 accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

20 Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5  
25 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body  
5 fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With  
10 respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

15 Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting  
20 protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ); luminescent labels, such as luminol; and  
fluorescent labels, such as fluorescein and rhodamine, and biotin.

25 One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which  
30 specifically binds to the polypeptide of interest; b) waiting for a time interval

the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

### *Diagnosis and Imaging*

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level,

pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of

use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g.,  
5 Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a  
10 pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the  
15 therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as  
20 liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH  
25 buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate,  
30 sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable

surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken  
5 to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp.  
10 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another  
15 embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During  
20 et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

25 Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic  
30 acid expression vector and administering it so that it becomes intracellular, e.g., by

composition of the invention, preferably a polypeptide or antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after



In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

10 In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. *Demonstration of Therapeutic or Prophylactic Activity*

15 The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

#### *Therapeutic/Prophylactic Administration and Composition*

20 The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical

to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection

transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which

acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect. :

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

5 For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art  
10 of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences  
15 encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment,  
20 nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In  
25 specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or  
30 indirect, in which case, cells are first transformed with the nucleic acids in vitro, then

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

## 25 *Gene Therapy*

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic

scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

5

### *Therapeutic Uses*

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed  
10 diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat,  
15 inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is  
20 not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the  
25 present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes  
30 without undue experimentation.

antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g.,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by



complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by  
5 reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1%  
10 Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in  
15 SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding  
20 immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein  
25 sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a  
30 secondary antibody (which recognizes the primary antibody, e.g., an anti-human

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### 5    ***Immunophenotyping***

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or  
10    maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning"  
15    with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to  
20    prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

### ***Assays For Antibody Binding***

25    The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions,  
30    gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays,

toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas  
5 Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGF (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-  
10 CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

15 Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp.  
20 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*,  
25 Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which  
30 is incorporated herein by reference in its entirety.

peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine  
5 fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

Further, an antibody or fragment thereof may be conjugated to a therapeutic  
10 moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin,  
15 dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine,  
20 thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic  
25 agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include,  
30 for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria

expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to  
5 identify antagonists of hIL-5. (See, Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred  
10 embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide  
15 tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used  
20 diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent  
25 materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics  
30 according to the present invention. Examples of suitable enzymes include horseradish

portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example,

5 Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053;

10 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entirety).

15 As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody

20 portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having

25 disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A

30 232,262). Alternatively, deleting the Fc part after the fusion protein has been

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody

Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, 1993, *TIB TECH* 11(5):155-215); and hygromycin, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), *Current Protocols in Human Genetics*, John Wiley & Sons, NY (1994); Colberre-Garapin et al., *J. Mol. Biol.* 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., *Mol. Cell. Biol.* 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA* 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.



glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell  
5 line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by  
10 appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection  
15 and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

20 A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively.  
25 Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-  
30 418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991);

*Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

5           In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo  
10   recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the  
15   ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate  
20   transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

          In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g.,  
25   cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which  
30   possess the cellular machinery for proper processing of the primary transcript,

BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*,  
5 and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for  
10 antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the  
15 generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding  
20 region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by  
25 adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus  
30 (AcNPV) is used as a vector to express foreign genes. The virus grows in

nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT  
5 Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an  
10 antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell  
15 for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with  
20 the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast  
25 expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid)  
30 containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO,

from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

#### *Methods of Producing Antibodies*

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a

Sons, NY, which are both incorporated by reference herein in their entireties ), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived

assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200  $\mu$ l cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

Add 50  $\mu$ l of the supernatant prepared by the protocol described in Example 31.

- 5 Incubate at 37 degree C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 37.

10 *Example 35: High-Throughput Screening Assay Identifying Neuronal Activity.*

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by polypeptide of the present invention.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by polypeptide of the present invention can be assessed.

- 25 The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:892)

5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:893)

- 30 Using the GAS:SEAP/Neo vector produced in Example 32, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified

product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2  
5 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells  
10 are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 31. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two  
15 months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

20 The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50 ul supernatant produced by Example 31, 37 degree C for 48 to 72 hr. As  
25 a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 37.

30 *Example 36: High-Throughput Screening Assay for T-cell Activity*

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of



agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF-KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF-KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I-KB is phosphorylated and degraded, causing NF-KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF-KB include IL-2, IL-6, GM-CSF, ICAM-1 and class I MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 31. Activators or inhibitors of NF-KB would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:894), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTC  
CATCCTGCCATCTCAATTAG:3' (SEQ ID NO:895)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:890)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCCATCTG  
CCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCCCCC  
CTAACTCCGCCCAGTTCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTAT  
TTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGG

AGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:896)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for  
5 mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-  
10 1 with Sall and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 33. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 33. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11,  
15 with a 5-10 fold activation typically observed.

#### *Example 37: Assay for SEAP Activity*

As a reporter molecule for the assays described in Examples 33-36, SEAP activity is  
20 assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer  
25 and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the  
30 chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the

results. An increase in chemiluminescence indicates reporter activity.

Reaction Buffer Formulation:

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25

-404

36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

---

*Example 38: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability*

5. Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.
- 10

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

- 15 For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The

adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37  
5 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10<sup>6</sup> cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then  
10 placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10<sup>6</sup> cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley Cell Wash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4.  
15 The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event  
20 caused by the a molecule, either polypeptide of the present invention or a molecule induced by polypeptide of the present invention, which has resulted in an increase in the intracellular Ca<sup>++</sup> concentration.

#### *Example 40: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity*

25

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of  
30 RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether polypeptide of the present invention or a molecule induced by polypeptide of the present invention is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 31, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate

is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by  
5 centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its  
10 ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

15 The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2</sub><sup>+</sup> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate  
20 the reaction mix at 30 degree C for 2 min. Initiate the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction  
25 mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

30 Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample

at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

*Example 41: High-Throughput Screening Assay Identifying Phosphorylation Activity*

5

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 40, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

15 Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 20 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 31 for 25 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 30 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-



resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by polypeptide of the present invention or a molecule induced by polypeptide of the present invention.

5     *Example 42: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation*

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

10         It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor  
15 (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since  
20 normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

25         Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to  $2.5 \times 10^5$  cells/ml. During this time, 100  $\mu$ l of sterile water is added to the  
30 peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and

in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10  $\mu$ l of prepared cytokines, 50  $\mu$ l of the supernatants prepared in Example 31 (supernatants at 1:2 dilution = 50  $\mu$ l) and 20  $\mu$ l of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100  $\mu$ l. The plates are then placed in a 37°C/5% CO<sub>2</sub> incubator for five days.

Eighteen hours before the assay is harvested, 0.5  $\mu$ Ci/well of [3H] Thymidine is added in a 10  $\mu$ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60  $\mu$ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

*Example 43: Assay for Extracellular Matrix Enhanced Cell Response (EMECR)*

The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the  $\alpha_5\beta_1$  and  $\alpha_4\beta_1$  integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of  $0.2 \mu\text{g}/\text{cm}^2$ . Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products of the invention (e.g., including, but not limited to, polynucleotides and polypeptides of the present invention, and supernatants produced in Example 31), are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO<sub>2</sub>, 7% O<sub>2</sub>, and 88% N<sub>2</sub>) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular polypeptide of the present invention is found to be a stimulator of

hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene encoding said polypeptide may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene  
5 product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow  
10 stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as,  
15 for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

20 *Example 44: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation*

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the  
25 proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors,  
30 which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNF $\alpha$  stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100  $\mu$ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5  $\mu$ g/ml hEGF, 5mg/ml insulin, 1 $\mu$ g/ml hFGF, 50mg/ml gentamycin, 50  $\mu$ g/ml Amphotericin B, 5%FBS. After incubation at 37°C for at least 4-5 hours, culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50 $\mu$ g/ml Amphotericin B, 0.4% FBS. Incubate at 37°C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed such that they always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Add 1/3 vol media containing controls or polypeptides of the present invention and incubate at 37°C/5% CO<sub>2</sub> until day 5.

Transfer 60 $\mu$ l from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4°C until Day 6 (for IL6 ELISA). To the remaining 100  $\mu$ l in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10 $\mu$ l). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100  $\mu$ l/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200  $\mu$ l/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50  $\mu$ l/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker. Plates are washed with wash buffer and blotted on

paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100  $\mu$ l/well. Cover the plate and incubate 1 h at RT. Plates are again washed with wash buffer and blotted on paper towels. Add 100  $\mu$ l/well of Enhancement Solution and shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each  
5 assay are tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the polypeptide of the present invention may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the polynucleotide/polypeptide  
10 of the present invention which gives a positive result. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the present invention and polynucleotides of the present invention may be used in wound healing and dermal regeneration, as well as the promotion of vasculogenesis, both of the blood vessels and lymphatics. The growth of  
15 vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides and polynucleotides of the invention may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign  
20 tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed  
25 wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's  
30 disease; and atherosclerosis. Moreover, antagonists of polypeptides and polynucleotides of the invention may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

5

*Example 45: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells*

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on  
10 lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the  
15 efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the  
20 cells and replaced with 100  $\mu$ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added to each well. Plates are held at 4°C  
25 for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10  $\mu$ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with  
30 PBS(+Ca,Mg) + 0.5% BSA. 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1

tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 ( $10^0$ ) >  $10^{-0.5}$  >  $10^{-1}$  >  $10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

*Example 46: Alamar Blue Endothelial Cells Proliferation Assay*

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM ) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C



incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

15 *Example 47: Detection of Inhibition of a Mixed Lymphocyte Reaction*

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

30 Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml, Organon Teknika

Corporation, West Chester, PA). PBMCs from two donors are adjusted to  $2 \times 10^6$  cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to  $2 \times 10^5$  cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate.

5 Dilutions of test materials (50  $\mu$ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1  $\mu$ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10  $\mu$ g/ml. Cells are cultured for 7-8 days at 37°C in 5% CO<sub>2</sub>, and 1  $\mu$ C of

10 [<sup>3</sup>H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes

15 and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

20 It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications,

25 journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties. Moreover, the hard copy of and the corresponding computer readable form of the

30 Sequence Listing of Serial No. 60/124,270 are also incorporated herein by reference in their entireties.

419

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209059
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input type="checkbox"/> For receiving Office use only This sheet was received with the international application Authorized officer	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer

**ATCC Deposit No.: 209059**

#### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209059**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

422

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209060</u>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")     	

For receiving Office use only	
<input type="checkbox"/>	This sheet was received with the international application
Authorized officer	

For International Bureau use only	
<input type="checkbox"/>	This sheet was received by the International Bureau on:
Authorized officer	

**ATCC Deposit No.: 209060**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209060**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.



425

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> . line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209061
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: 209061**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209061**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

428

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209062
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: 209062**

#### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209062****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

431

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209063</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

ATCC Deposit No.: 209063

#### CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.



**ATCC Deposit No.: 209063**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

434

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209064
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: 209064**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: 209064

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

437

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209065</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

For receiving Office use only	
<input type="checkbox"/>	This sheet was received with the international application
Authorized officer	

For International Bureau use only	
<input type="checkbox"/>	This sheet was received by the International Bureau on:
Authorized officer	

**ATCC Deposit No.: 209065**

#### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209065**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

440

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 20 May 1997	Accession Number 209066
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

For receiving Office use only
<input type="checkbox"/> This sheet was received with the international application
Authorized officer

For International Bureau use only
<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer



ATCC Deposit No.: 209066

#### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209066**

### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

443

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209067</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: 209067**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: 209067

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

446

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209068</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div>For receiving Office use only</div> <div><input type="checkbox"/> This sheet was received with the international application</div> <div>Authorized officer</div>	<div>For International Bureau use only</div> <div><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div>Authorized officer</div>

**ATCC Deposit No.: 209068**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209068**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.



449

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>20 May 1997</u>	Accession Number <u>209069</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: 209069**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209069**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

452

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u> .	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 12 January 1998	Accession Number 209579
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<b>For receiving Office use only</b>	<b>For International Bureau use only</b>
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

ATCC Deposit No.: 209579

#### CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209579**

### **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

455

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>12 January 1998</u>	Accession Number <u>209578</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: 209578**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.



**ATCC Deposit No.: 209578**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

458

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>16 July 1998</u>	Accession Number <u>203067</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="text-align: center;">For receiving Office use only</div> <div><input type="checkbox"/> This sheet was received with the international application</div> <div>Authorized officer</div>	<div style="text-align: center;">For International Bureau use only</div> <div><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div>Authorized officer</div>

ATCC Deposit No.: 203067

### CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: 203067

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

461

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>16 July 1998</u>	Accession Number <u>203068</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: 203068**

## **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

## **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

## **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

## **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: 203068

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

464

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 01 February 1999	Accession Number 203609
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input type="checkbox"/> For receiving Office use only This sheet was received with the international application Authorized officer	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer



ATCC Deposit No.: 203609

#### CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 203609****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

467

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 01 February 1999	Accession Number 203610
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer

ATCC Deposit No.: 203610

#### CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: 203610

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

470

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
---	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>17 November 1998</u>	Accession Number <u>203485</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: 203485**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 203485**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.



473

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b>	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>18 June 1999</u>	Accession Number <u>PTA-252</u>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable)         This information is continued on an additional sheet <input type="checkbox"/>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	
<b>For receiving Office use only</b> <input type="checkbox"/> This sheet was received with the international application Authorized officer	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer

**ATCC Deposit No.: PTA-252**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-252****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PA104PCT	International application No.	UNASSIGNED
--	----------	-------------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>66</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>18 June 1999</u>	Accession Number <u>PTA-253</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: PTA-253**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-253****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

479

Applicant's or agent's file reference number	PA104PCT	International application?	UNASSIGNED
---	----------	----------------------------	------------

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>86</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>22 December 1999</u>	Accession Number <u>PTA-1081</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u> In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No.: PTA-1081**

### **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

### **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

### **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

### **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.



**ATCC Deposit No.: PTA-1081**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

*What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:
- 5 (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
- 10 (c) a polynucleotide encoding a polypeptide fragment of a polypeptide encoded by SEQ ID NO:X or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
- (d) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
- 15 (e) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;
- 20 (f) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X, having biological activity;
- (g) a polynucleotide which is a variant of SEQ ID NO:X;
- 25 (h) a polynucleotide which is an allelic variant of SEQ ID NO:X;
- (i) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
- (j) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide
- 30

sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a protein.

5

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

10

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

15

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

20

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

25

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

30

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.
11. An isolated polypeptide comprising an amino acid sequence at least  
5 95% identical to a sequence selected from the group consisting of:
- (a) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
  - (b) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone, having biological activity;
  - 10 (c) a polypeptide domain of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
  - (d) a polypeptide epitope of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;
  - (e) a full length protein of SEQ ID NO:Y or of the sequence encoded by the  
15 cDNA included in the related cDNA clone;
  - (f) a variant of SEQ ID NO:Y;
  - (g) an allelic variant of SEQ ID NO:Y; or
  - (h) a species homologue of the SEQ ID NO:Y.
- 20 12. The isolated polypeptide of claim 11, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.
13. An isolated antibody that binds specifically to the isolated polypeptide  
25 of claim 11.
14. A recombinant host cell that expresses the isolated polypeptide of claim 11.
- 30 15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

5           16.    The polypeptide produced by claim 15.

17.    A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

10

18.    A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

15       (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19.    A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

20       (a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

25       20.    A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

30

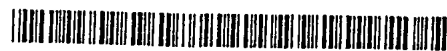
21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

- 5       (a) expressing SEQ ID NO:X in a cell;  
      (b) isolating the supernatant;  
      (c) detecting an activity in a biological assay; and  
      (d) identifying the protein in the supernatant having the activity.

10       23. The product produced by the method of claim 20.

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
21 September 2000 (21.09.2000)

PCT

(10) International Publication Number  
WO 00/55180 A3

(51) International Patent Classification<sup>7</sup>: A01N 43/04,  
C07H 21/02, 21/04, C12Q 1/68

(74) Agents: WALES, Michele, M. et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).

(21) International Application Number: PCT/US00/05918

(22) International Filing Date: 8 March 2000 (08.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/124,270 12 March 1999 (12.03.1999) US

(81) Designated States (*national*): AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— With international search report.

(88) Date of publication of the international search report:  
18 January 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Laytonsville, MD 20882 (US).



WO 00/55180 A3

(54) Title: HUMAN LUNG CANCER ASSOCIATED GENE SEQUENCES AND POLYPEPTIDES

(57) Abstract: This invention relates to newly identified lung or lung cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "lung cancer antigens", and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such lung cancer antigens for detection, prevention and treatment of disorders of the lung, particularly the presence of lung cancer. This invention relates to the lung cancer antigens as well as vectors, host cells, antibodies directed to lung cancer antigens and recombinant and synthetic methods for producing the same. Also provided are diagnostic methods for diagnosing and treating, preventing and/or prognosing disorders related to the lung, including lung cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of lung cancer antigens of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/05918

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01N 43/04; C07H 21/02, 21/04; C12Q 1/68

US CL : 435/06; 514/44; 536/23.1, 24.3

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/06; 514/44; 536/23.1, 24.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GERHOLD et al. It's the genes! EST access to human genom content. BioEssays. 1996, Vol. 18, No. 12, pages 973-981.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

23 MAY 2000

Date of mailing of the international search report

19 OCT 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231  
Facsimile No. (703) 305-3230

Authorized officer

MICHAEL BORIN

Telephone No. (703) 308-4156



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/05918

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-10, SEQ ID No.:1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/05918

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional examination fees must be paid.

Group I, claims 1-10, drawn to nucleic acids, vectors, host cells and method of making recombinant cell using said nucleic acids.

Group II, claims 11, 12 and 16 drawn to isolated polypeptides.

Group III, claim 13, drawn to antibody.

Group IV, claim 14, drawn to a host cell expressing polypeptide of Group II.

Group V, claim 15, drawn to method of making polypeptide.

Group VI, claim 17, drawn to method for polypeptide-based method of treatment.

Group VII, claim 18, drawn to polynucleotide-based method of diagnosing a pathological condition.

Group VIII, claim 19, drawn to polypeptide-based method of diagnosing a pathological condition.

Group IX, claim 20, drawn to polypeptide-based method of identifying a binding partner to the polypeptide and to a product determined by this method.

Group XI, claim 21, drawn to a gene.

Group XII, claims 22 and 23, drawn to polynucleotide-based method of identifying activity.

The inventions listed as Groups I-XII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The inventions listed as Groups I-IV, XI are drawn to different products which they lack the same or corresponding special technical features. Groups VII, XII are different methods of use of the product of Group I. Groups VI, VIII, IX are different methods of use of the product of Group II. Group V is method of use of product of Group IV.

#### *Sequence Election Requirement Applicable to All Groups*

In addition, each Group detailed above reads on distinct Groups drawn to multiple sequences. The sequences are distinct because they are unrelated sequences, and a further lack of unity is applied to each Group. The lack of unity is partially waived and the Applicants must further elect 10 sequences for examination in the elected Group detailed above. Payment of fees for an additional invention will entitle the Applicants to examination of four additional sequences.

tcccttcata ttaatatattg ataaagattt taatttatgt aaaacttcta aagcagaatc 660  
aaagctcctc ttggggaaat ggcaagtott taggataggc aagaccctgt atgaatagta 720  
ccaaagcatt accgcatggt agagaacaca ctcgattaaa aatgttaagc tatctgaaaa 780  
ataaaatgtg caagtcttca ggatggcaca aaacaaaggt taatgcttct tggggcacat 840  
ttcttagagg gcttgctgag tgtgtaaata taatcgactt ttgtttgtgt tacatgactt 900  
ctgtgacttc attgaaaatc tgcacaattc agtttcagct ctggattact tcagttgacc 960  
tttgtgaagg tttttatctg tgtagaatgg gtgtttgact tgttttagcc tattaattt 1020  
ttattttctt tcaactctgta ttaaaagtaa aacttactaa aagaaaagag gtttgtgttc 1080  
acattaaatg gttttgggtt ggcttctttt agtcaggctt tctgaacatt gagatctct 1140  
gaacttagag ctcttcaatc ctaagatttt catgaaaagc ctctcacttg aacccaaacc 1200  
agagtactct tactgcctct tttctaattg ttcaggaaaa gcattgccag ttcagtcttt 1260  
tcaaaatgag ggagaaacat ttgctgcctt tgaataaca agactcagtg cttatttttt 1320  
aaactgcatt ttaaaaattg gatagtataa taacaataag gagtaagcca ctttttatag 1380  
gcacctgta gttttatagt tcttaatcta aacattttat atttccttct tttggaaaaa 1440  
acctwcacgc tayaagccac catatgcaca gactatacag tgagttgagt gggctctcca 1500  
cagctcttga ggggattacn aagtcgcca tatcaccccc gngtattgga aggattttng 1560  
aattgggcga tggggggaaa caaaggnccc ccccg 1596

&lt;210&gt; 156

&lt;211&gt; 1654

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 156

atgaagaaac tgaggccctg tgatgtgaag tgacttgccc cccagccaca cagcwgacc 60  
attctggctg ctgtctggac aagaagtcgt agggggtgag ggtggaagct gggaaaccca 120  
caggaggcaa ccacactagt ttagctggcc catggcagtc ccactggccg catcgagggc 180  
ttcaccaacg tcaaggagct gtatggcaag atcgccgagg ccttccgcct gccaaactgcc 240  
gaggtgatgt tctgcacct gaacaccac aaagtgkaca tggacaagct cctggggggc 300  
cagatcgggc tggaggactt catcttcgcc cacgtgaagg ggcagcgcaa ggaggtggag 360  
gtgttcaagt cggaggatgc actcgggctc accatcacgg acaacggggc tggctacgcy 420  
ttcatcaago gcatcaagga gggcagcgtg atcgaccaca tccacctcat cagcgtgggc 480  
gacatgatcg aggcattaa cgggcagagc tgctgggctg ccggcactac gaggtggccc 540  
ggctgctcaa ggagctgccc cgaggccgta ccttcacgct gaagctcacg gagcctcgca 600  
aggccttcga catgatcagc cagcgttcag cgggtggccg ccctggctct ggcccacaac 660  
tgggcastgg ccgagggacc ctgcggctcc gatcccgggg ccccgccacg gtggaggatc 720  
tgccctctgc ctttgaagag aaggccattg agaaggtgga tgacctgctg gagagttaca 780  
tgggtatcag ggacacggag ctggcgccca ccatggtgga gctgggaaag gacaaaagga 840  
accgggatga gctggccgag gccctggacg aacggctggg tgactttgcc ttccctgacg 900  
agttcgtctt tgacgtctgg ggcgccattg gggacgcaa ggtcggccgc tactaggact 960  
gcccccgac cctgcgatga tgaccgggc gnaacctggt gggggccccc agcagggaca 1020  
ctgacgtcag gaccgagcc tccagcctga gcctagctca gcagcccaag gacgatggtg 1080  
aggggaggtg gggccaggcc cctgccccg ctccaatcgg taccatcccc tccctggttc 1140  
ccagtctgga cggggtcccc ggccccctg tgccctgttc cccacctacc tcagctgggt 1200  
caggcacagg gaggggagg atcagccaaa ttggggggcc acccccgctt ccaccacttt 1260  
ccaccatcag ctgccaactt ggtccctctg tctccctggg gcottgggtt ctgtttgggg 1320  
gtcatgacct tcctagtctt ctgacgcagg gaatacagg gagagggttg tcttccccc 1380  
cagcaaatgc aataatgcc taccacctcc tgagaggagc cccctccctg tggagcctgt 1440  
tacctccgca tttgacaga gtctgctgtg aaccccgcaa cctcctcccc acctcccate 1500  
tctcctcca ggccatccc tggccagag caggaggag ggagggacga tggcggtggg 1560  
ttttgtatc tgaatttgct gtcttgaaca taaagaatct atctgctgtt aaaaaaaaaa 1620



aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaag

1654

<210> 157  
<211> 1815  
<212> DNA  
<213> Homo sapiens

<220>  
<221> misc feature  
<222> (1808)  
<223> n equals a,t,g, or c

<400> 157  
tcgacccacg cgtccggggc ttccgggggc gctcaggctc cggggcgccct aggcctgggt 60  
tgtcctttgc atctgcacgt gtctgcagtc gtttccgcga tgetgcctct gctgcgctgc 120  
gtgccccgtg tgctgggctc ctccgtcgcc ggccctccgcg ctgcccggcc cgcctcgccct 180  
ttccggcagc tcctgcagcc ggcaccccggt ctgtgcaccc ggcccttcgg gctgctcagc 240  
gtgcgcgcag gtcccgagcg gcggccgggc ctccctgcggc ctgcgggacc ctgcgcctgt 300  
ggctgtggct gcggctcgct gcacaccgac ggagacaaag cttttgttga ttccctgagt 360  
gatgaaatta aggaggaaaag aaaaattcag aagcataaaa ccctccctaa gatgtctgga 420  
ggttggggagc tggaactgaa tgggacagaa gcgaaattag tgcggaaagt tgccggggaa 480  
aaaatcacgg tcactttcaa cattaacaac agcatccac caacatttga tggtagggag 540  
gaaccctcgc aagggcagaa ggttgaagaa caggagcctg aactgacatc aactcccaat 600  
ttcgtggttg aagttataaa gaatgatgat ggcaagaagg cccttgtgtt ggactgtcat 660  
tatccagagg atgaggttg acaagaagac gaggctgaga gtgacatctt ctctatcagg 720  
gaagttagct ttcagtcac tggcgagtct gaatggaagg atactaatta tacactcaac 780  
acagattcct tggactgggc cttatatgac cacctaattg atttccttgc cgaccgaggg 840  
gtggacaaca cttttgcaga tgagctggtg gagctcagca cagccctgga gcaccaggag 900  
tacattactt ttcttgaaag cctcaagagt tttgtcaaga gccagtagag cagacagatg 960  
ctgaaagcca tagttcatg gcaggctttg gccagtgaac aaatcctact ctgaagctag 1020  
acatgtgctt tgaatgatt atcatcctaa tatcatgggg gaaaaaatac caaattttaa 1080  
ttatatgttt tgtgttctca ttattatca tttttttctg taaaaatota ttatttctag 1140  
atttttgtat aacatgatag acataaaaatt ggtttatctc ctccaaggca gtttgtcttt 1200  
ttctattcct ccccttcaa cctgygtcac aaaagaccaa gaacagatgt cggaaaagtt 1260  
tttttttctt cagtattgtt taaaagtttc aatacaaaaat aagttataaa taaaaggctt 1320  
gtatgtacaa ggctcctcag agggaaatgag ttgtcttcaa ccccatagaa tgatgtgagt 1380  
ccaagctggc tctagaggat cacagcccaa gtatcacagg ccttgsttga tcagctcctg 1440  
ttgaatttcc tccagcacag ccatgtctat cagctcctcc arctgagcca agtcttctgg 1500  
acaattctcc actgactgca aagcattcca ctcttcttcc atcactctt gaactagaaa 1560  
gctgttctga gaattccctg gccactgct tccagctggc ggtacctgt taggagcctg 1620  
tcccggctgt ttctcattct ctccaggcat ctctgccgga aagcctcttt ccaaggcggc 1680  
gagccacca gtttgtacag ggagcggcgc ggagacctca acgactccgc catctcctct 1740  
tcgcgggaga caaagccaca agaccggtc cctggaggcg cggcacagac ccctgggagg 1800  
tgtatgcnc cgggt

1815

<210> 158  
<211> 1397  
<212> DNA  
<213> Homo sapiens

&lt;220&gt;



603

100

105

110

Ala Thr Trp  
115

&lt;210&gt; 598

&lt;211&gt; 99

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 598

Ala Arg Pro Cys Met Asn Ser Thr Lys Ala Leu Pro His Gly Arg Glu  
1 5 10 15

His Thr Arg Leu Lys Met Leu Ser Tyr Leu Lys Asn Lys Met Cys Lys  
20 25 30

Ser Ser Gly Trp His Lys Thr Lys Val Asn Ala Ser Trp Gly Thr Phe  
35 40 45

Leu Arg Gly Leu Ala Glu Cys Val Asn Ile Ile Asp Phe Cys Leu Cys  
50 55 60

Tyr Met Thr Ser Val Thr Ser Leu Lys Ile Cys Thr Ile Gln Phe Gln  
65 70 75 80

Leu Trp Ile Thr Ser Val Asp Leu Cys Glu Gly Phe Tyr Leu Cys Arg  
85 90 95

Met Gly Val

&lt;210&gt; 599

&lt;211&gt; 151

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (56)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 599

Arg Ala Glu Leu Leu Gly Cys Arg His Tyr Glu Val Ala Arg Leu Leu  
1 5 10 15

Lys Glu Leu Pro Arg Gly Arg Thr Phe Thr Leu Lys Leu Thr Glu Pro





604

20	25	30
Arg Lys Ala Phe Asp Met Ile Ser Gln Arg Ser Ala Gly Gly Arg Pro		
35	40	45
Gly Ser Gly Pro Gln Leu Gly Xaa Gly Arg Gly Thr Leu Arg Leu Arg		
50	55	60
Ser Arg Gly Pro Ala Thr Val Glu Asp Leu Pro Ser Ala Phe Glu Glu		
65	70	75 80
Lys Ala Ile Glu Lys Val Asp Asp Leu Leu Glu Ser Tyr Met Gly Ile		
85	90	95
Arg Asp Thr Glu Leu Ala Ala Thr Met Val Glu Leu Gly Lys Asp Lys		
100	105	110
Arg Asn Pro Asp Glu Leu Ala Glu Ala Leu Asp Glu Arg Leu Gly Asp		
115	120	125
Phe Ala Phe Pro Asp Glu Phe Val Phe Asp Val Trp Gly Ala Ile Gly		
130	135	140
Asp Ala Lys Val Gly Arg Tyr		
145	150	

<210> 600  
 <211> 315  
 <212> PRT  
 <213> Homo sapiens

<400> 600  
 Ser Thr His Ala Ser Gly Ala Ser Gly Gly Ala Gln Val Ala Gly Arg  
 1 5 10 15  
 Leu Gly Leu Gly Cys Pro Leu His Leu His Val Phe Ala Val val Ser  
 20 25 30  
 Ala Met Leu Pro Leu Leu Arg Cys Val Pro Arg Val Leu Gly Ser Ser  
 35 40 45  
 Val Ala Gly Leu Arg Ala Ala Ala Pro Ala Ser Pro Phe Arg Gln Leu  
 50 55 60  
 Leu Gln Pro Ala Pro Arg Leu Cys Thr Arg Pro Phe Gly Leu Leu Ser  
 65 70 75 80  
 Val Arg Ala Gly Ser Glu Arg Arg Pro Gly Leu Leu Arg Pro Arg Gly  
 85 90 95

